ANTIDIABETIC AND TOXICITY ASSESSMENT OF COMBINATION OF PIPER NIGRUM AND ARTOCARPUS HETROPYLLUS

A Dissertation submitted to
THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

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Under the guidance of

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OCTOBER 2016

Certificate

This is to certify that the dissertation work entitled **"ANTIDIABETIC AND TOXICITY ASSESSMENT OF COMBINATION OF *PIPER NIGRUM* AND *ARTOCARPUS HETROPYLUS*"** is a bonafide work done by **MOHAMMED HALEEL.P.M (Reg No: 261425663)** under my guidance for the partial fulfillment for the award of degree of Master of Pharmacy in Pharmacology, at Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, affiliated to The Tamilnadu Dr. M.G.R Medical University, Chennai.

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INTERNAL EXAMINER **EXTERNAL EXAMINER**

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Declaration

I hereby declare with immense pleasure and satisfaction that this dissertation work entitled "**ANTIDIABETIC AND TOXICITY ASSESSMENT OF COMBINATION OF *PIPER NIGRUM* AND *ARTOCARPUS HETROPYLUS*"** was carried out by me under the guidance of Dr. C.SENTHIL KUMAR M.Pharm., PGDCR., PhD. lecturer Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore.

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ACKNOWLEDGEMENT

First of all, I would like to extend my sincere gratitude to God Almighty, whose divine intervention was instrumental in the successful completion of this project work. There are a number of people whom I consider of this simple anthology and to whom I owe so much.

I would like to express my whole hearted gratitude to **MY FAMILY** and **MY FRIENDS** without whose support and blessings, this endeavor would not have been completed.

It is a great pleasure to acknowledge my sincere and deep sense of gratitude to my respected guide **Dr. C.SENTHIL KUMAR M. Pharm, PGDCR, PhD** of department of pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, for his invaluable guidance, help, suggestions and the confidence that he has shown in me throughout the course of my work.

I express my sincere gratitude to **Dr. D. BENITO JOHNSON M.Pharm, PhD** Department of Pharmacology**,** RVS College of pharmaceutical sciences, Sulur, Coimbatore, for his help and guidance throughout the completion of my work.

I sincerely thank **Dr. R. VENKATANARAYANAN, M.Pharm., Ph.D, Principal**, RVS College of pharmaceutical sciences, Sulur, Coimbatore, for his inspirations and being a great facilitator.

I am grateful to **Mr. SAKTHIVEL, Mr.SURESH, Dr. W.D. SAM SOLOMON, Miss.SATHYA, Miss. ASHWINI** and all the teachers and non teachers of my college for their meticulous guidance and encouragement provided to me for the completion of my dissertation work.

However, it would not have been possible to complete my work without kind support and help of all my friends especially **RASHID.K, SHRUTI RAMESH TIMANE, BANSHONGDOR H MAWLIEH, TIMAI PASSAH, SANDEEP.M, SABITH, ROLLY.**

As a final word, I would like to extend my sincere thanks to each and every individual who have been a source of support and helped me to complete my dissertation work successfully.

MOHAMMED HALEEL P.M

ABSTRACT

In the present study the combination of ethanolic leaf extract of *Piper nigrum* and *Artocarpus hetrophyllus* in the ratio 1:1 were administered orally to study the toxicity and antidiabetic effects.

Acute toxicity studies on the albino rats show no morality at a dose of 2000mg/kg, during a time period of 14 days. NOAEL were not seen in the entire study period

In-vivo sub-acute oral toxicity study were performed to evaluate the toxicities of 28 days by continuous administration of the prepared extract and no animal mortalities were observed in any of the study groups throughout the study period For all the dose level falling in the category NOAEL <100 mg/kg. The observations indicated that long-term administration of the extract had no adverse effects on the general health of the animals. No significant differences were observed in body weights or food consumption of the animals. Hence this preparation can be utilized clinically.

The animals were induced diabetic with STZ at a dose of 60mg/kg intraperatoneal and the diabetic animals were treated with extract at a dose of 200mg/kg along with standard drug glibenclanide for 28 days orally. The serum glucose, body weight were measured which showed significantly increase when compared with positive control group.

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LIST OF ABBREVIATIONS

DM	Diabetes Mellitus
IAPP	Islet Amyloid Polypeptide
MHC	Major Histocompatibility Complex
HLA	Human Leucocyte Antigens
GAD	Glutamic Acid Decarboxylase
T1DM	Type 1 Diabetes Mellitus
GLUT 4	Glucose Transporter
IDDM	Type 1 Diabetes Mellitus
LPL	Lipoprotein Lipase
ICAs	Islet Cell Autoantibodies
MODY	Maturity Onset Diabetes Of The Young
PCOS	Polycystic Ovary Syndrome
DKA	Diabetes Ketoacidosis
HHS	Hyperglycemic Hyperosmolar State
STZ	Streptozotocin
NOAEL	No Observational Adverse Effect Level

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DIABETES MELLITUS

1. INTRODUCTION

The Diabetes Mellitus is being one of five leading causes of deaths and debilitating disease in the world. One hundred and fifty million people were suffering from diabetes wide reaching, which is almost five times more than the estimates one decade ago and it may double in the year 2030 [1]. The development of diabetic complications is a major cause of morbidity and mortality and is an ever-increasing burden to healthcare authorities in both developed and developing nations. Epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progress of diabetic complications [2].

Diabetes mellitus or simply diabetes is a chronic metabolic disorder of carbohydrate, lipid and protein metabolism characterized by hyperglycemia, glycosurea, hyperlipidemia, negative nitrogen balance and sometimes ketonemia due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action [3]. The hallmark of diabetes mellitus is polyuria-excessive urine production**,** polydipsia-excessive thirst and polyphagia-excessive eating [4].

Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life[5].

The pathogenesis of diabetes mellitus and its complications is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides [6]. However, on chronic usage most of these agents produced several side effects, including hypoglycemic coma, insulin resistance, hyper-sensitivity, cholesterol, jaundice, abdominal pain, anorexia and metallic taste [4].

For various reasons in recent years, the popularity of herbal medicines in diabetic control has increased. Natural plant drugs are frequently considered to be less toxic with lower side effects

than synthetic ones [7].Therefore searching herbal product with anti diabetic activity possessing fewer side effects receives considerable publicity and provides an opportunity to cure this disease [8].

EPIDEMIOLOGY

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. Based on current trends, the International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030 (Fig. 1). Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is rising much more rapidly, presumably because of increasing obesity, reduced activity levels as countries become more industrialized, and the aging of the population. In the most recent estimate for the United States (2010), the Centers for Disease Control and Prevention (CDC) estimated that 25.8 million persons, or 8.3% of the population, had diabetes (27% of the individuals with diabetes were undiagnosed). Approximately 1.6 million individuals (>20 years) were newly diagnosed with diabetes in 2010. DM increases with aging. In 2010, the prevalence of DM in the United Sates was estimated to be 0.2% in individuals aged <20 years and 11.3% in individuals aged >20 years. In individuals aged >65 years, the prevalence of DM was 26.9%. The prevalence is similar in men and women throughout most age ranges (11.8% and 10.8%, respectively, in individuals aged >20 years). Worldwide estimates project that in 2030 the greatest number of individuals with diabetes will be aged 45–64 years. [9]

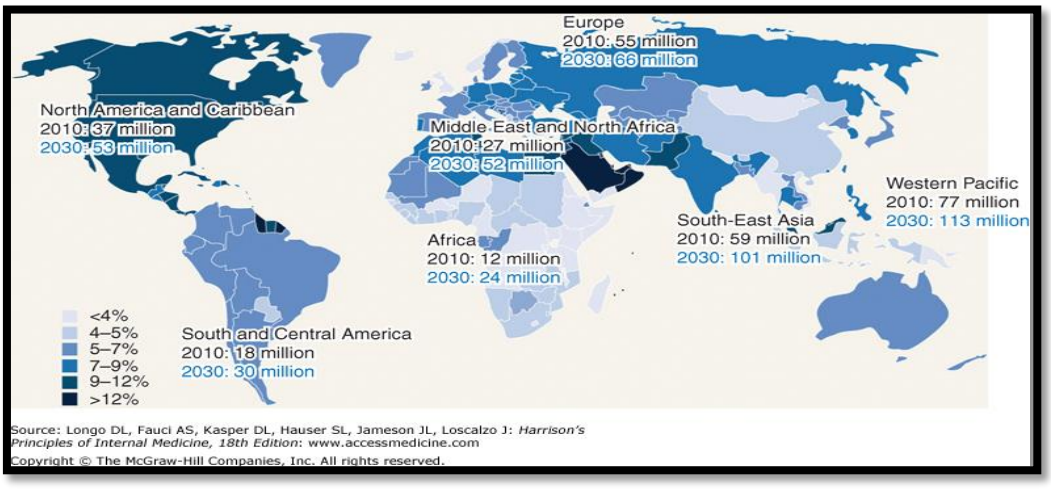


Figure 1:-*Worldwide prevalence of diabetes mellitus.* *Comparative prevalence (%) of estimates of diabetes (20–79 years), 2010*

Presently as many as 50% of people with diabetes are undiagnosed. Since therapeutic intervention can reduce complications of the disease, there is a need to detect diabetes early in its course. The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity. Its incidence is increasing rapidly, and by 2030 this number is estimated to almost around 552 million [10, 11]. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries, where the majority of patients are aged between 45 and 64 years. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 [11] (Table 1). It is projected that the latter will equal or even exceed the former in developing nations, thus culminating in a double burden as a result of the current trend of transition from communicable to non-communicable diseases [12,13]

	2000		2030	
Ranking	Country	People with diabetes (millions)	Country	People with diabetes (millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian Federation	4.6	Bangladesh	11.1
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	Bangladesh	3.2	Egypt	6.7

Table 1:- *List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030[11].*

CLASSIFICATION OF DIABETES MELLITUS

The classification of diabetes mellitus has been a major discussion point over the last few years. It has been increasingly recognized that the old classification system based upon a patient's dependence on insulin was misleading; under the old system patients were either classified as

- Insulin Dependent Diabetes Mellitus (IDDM)
- Non - Insulin Dependent Diabetes Mellitus (NIDDM).

In 1998, a new classification system based upon the etiological factors at work in diabetes was proposed by the WHO and it is listed below: this has now become the accepted system for classifying diabetes mellitus [14].

ETIOLOGIC CLASSIFICATION OF DIABETES MELLITUS

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class [23]

I. Type 1 Diabetes Mellitus	➢ ***Autoimmune*** ➢ ***Idiopathic***
II. Type 2 Diabetes Mellitus	➢ ***Relative Insulin Deficiency*** ➢ ***Disorders Of Insulin Secretion*** ➢ ***Insulin Resistance***
III. Other Specific Types Of Diabetes Mellitus	➢ ***Genetic Defects In β-Cell Function*** ** Chromosome 12, HNF-1α (MODY 3)* ** Chromosome 7, glycosidase (MODY 2)* ** Chromosome 20, HNF-4α (MODY 1)* ** Mitochondrial DNA* ** Monogenic diabetes*

	➢ ***Genetic Defects In Insulin Action*** * *Type A insulin resistance* * *Leprechaunism* * *Rabson-Mendenhall syndrome* * *Lipotrophic diabetes* ➢ ***Disease Of The Exocrine Pancreas*** * *Pancreatitis* * *Pancreatectomy/trauma* * *Neoplasia* * *Cystic fibrosis* * *Hemochromatosis* * *Fibrocalcific pancreatopathy* ➢ ***Endocrinopathies*** * *Acromegaly* * *Cushing syndrome* * *Glucagonoma* * *Pheochromocytoma* * *Hyperthyroidism* * *Somatostatinoma* * *Aldosteronoma* ➢ ***Pharmacologically/Chemically Induced*** * *Vacor* * *Pentamidine* * *Nicotinic acid* * *Glucocorticoids* * *Thyroid hormones* * *β-adrenergic agonists* * *Tiazides* * *α interferon* ➢ ***Infections***

	* *Congenital rubeola* * *Cytomegalovirus* ➢ ***Infrequent Forms Of Autoimmune Diabetes*** * *Stiff-man syndrome)* * *Antibodies against insulin receptors* ➢ ***Other Syndromes Occasionally Associated With Diabetes*** * *Down syndrome* * *Klinefelter syndrome* * *Turner syndrome* * *Wolfram syndrome* * *Friedreich ataxia* * *Huntington's chorea* * *Lawrence-Moon-Biedel syndrome* * *Myotonic dystrophy* * *Porphyria* **Prader-Willi syndrome*
IV. Gestational diabetes mellitus	***Occurs in mostly in women during gestation***.

Table 2:- *Etiologic Classification of Diabetes Mellitus. Adapted from WHO and ADA* [17, 18, 13]

i. Type 1 diabetes mellitus:

An autoimmune disease in which the immune system mistakenly destroys the insulin-making beta cells of the pancreas. It typically develops more quickly than other forms of diabetes. It is usually diagnosed in children and adolescents, and sometimes in young adults. To survive, patients must administer insulin medication regularly. Type 1diabetes used to be called **juvenile diabetes** and **insulin-dependent diabetes mellitus (IDDM)**. However, those terms are not accurate because children can develop other forms of diabetes, adults sometimes develop type 1, and other forms of

diabetes can require insulin therapy. A variation of type 1 that develops later in life, usually after age 30, is called latent autoimmune diabetes of adulthood (LADA). Sometimes patients with autoimmune diabetes develop insulin resistance because of weight gain or genetic factors. This condition is known as double diabetes.

ii. Type 2 diabetes mellitus

A disorder of metabolism, usually involving excess weight and insulin resistance. In these patients, the pancreas makes insulin initially, but the body has trouble using this glucose-controlling hormone. Eventually the pancreas cannot produce enough insulin to respond to the body's need for it. Type 2 diabetes is by far the most common form of diabetes, accounting for 85 to 95% of cases in developed nations and an even higher percentage in developing nations, according to the International Diabetes Federation. This disease may take years or decades to develop. It is usually preceded by pre diabetes, in which levels of glucose (blood sugar) are above normal but not high enough yet for a diagnosis of diabetes. People with pre diabetes can often delay or prevent the escalation to type 2 diabetes by losing weight through improvements in exercise and diet, as the Diabetes Prevention Program and other research projects have demonstrated. Type 2 diabetes used to be called **adult-onset diabetes** and **non-insulin-dependent diabetes mellitus (NIDDM)**. Those terms are not accurate because children can also develop this disease, and some patients require insulin therapy.

iii. Other specific type (Monogenic diabetes)

Diabetes caused by another condition. The many potential sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis to medical treatments including corticosteroids, other immunosuppressives, diuretics and pancreatectomy.

iv. Gestational Diabetes Mellitus (GDM):

A temporary metabolic disorder that any previously nondiabetic woman can develop during pregnancy, usually the third trimester. Hormonal changes contribute to this disease, along with excess weight and family history of diabetes. About 4% of pregnant women develop gestational diabetes, according to the American Diabetes Association. [15, 16]

Blood Sugar Classification	Fasting Blood Sugar Levels	Post Meal Blood Sugar Levels
Normal	70-100 mg/dL	70-140 mg/dL
Prediabetes	101-125 mg/dL	141-200 mg/dL
Diabetes	125 mg/dL and above	200 mg/dL and above

Figure 2:- *Blood Sugar Levels*

The type of diabetes is based on the presumed etiology. This table provides information about the two most common types of diabetes: type 1 and type 2 diabetes (**Table 3**). [19, 20]

	Type 1	**Type 2**
Age	Childhood	Pubertal
Onset	Acute; severe	Mild-severe; often insidious
Insulin secretion	Very low	Variable
Insulin sensitivity	Normal	Decreased
Insulin dependence	Permanent	Temporary; may occur later
Racial/ethnic groups at increased risk	All (low in Asians)	African Americans, Hispanics, Native Americans, Asian/Pacific Islanders
Genetics	Polygenic	Polygenic
Proportion of those with diabetes	80%	10%-20%
Association: obesity	No	Strong
Acanthosis nigricans	No	Yes
Autoimmune etiology	Yes	No

Table 3:- *Characteristic features of Type 1 and Type 2 Diabetes*

INSULIN BIOSYNTHESIS, SECRETION, AND ACTION [21]

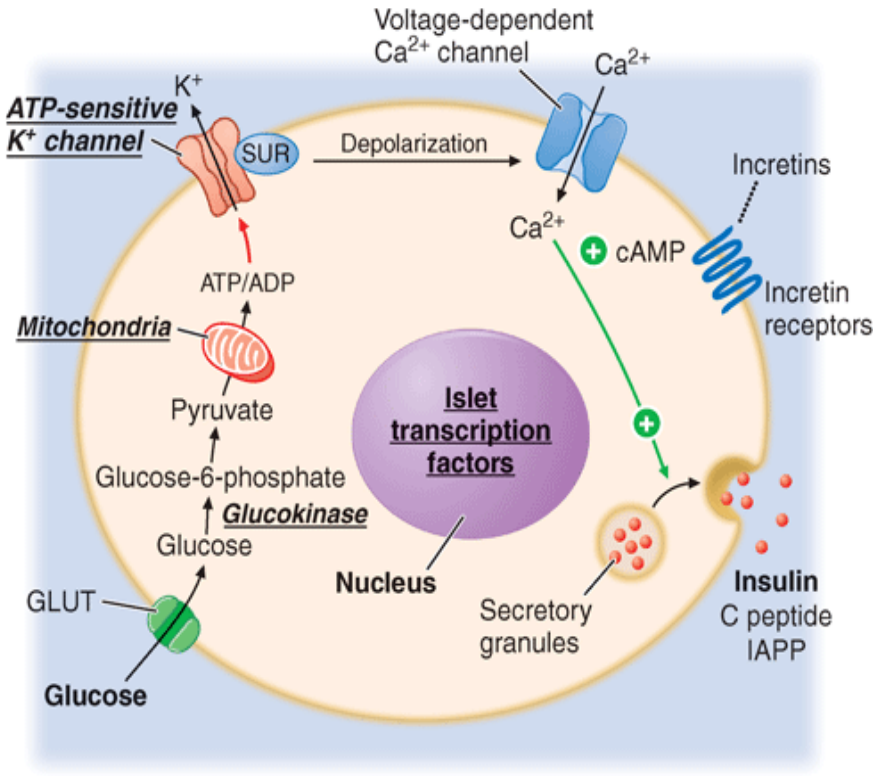
Biosynthesis

Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as a single chain 86-amino-acid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the aminoterminal signal peptide, giving rise to proinsulin. Proinsulin is structurally related to insulin-like growth factors I and II, which bind weakly to the insulin receptor. Cleavage of an internal 31-residue fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells. Because C peptide is cleared more slowly than insulin, it is a useful marker of insulin secretion and allows discrimination of endogenous and exogenous sources of insulin in the evaluation of hypoglycemia. Pancreatic beta cells co-secrete islet amyloid polypeptide (IAPP) or amylin, a 37-amino-acid peptide, along with insulin. The role of IAPP in normal physiology is incompletely defined, but it is the major component of the amyloid fibrils found in the islets of patients with type 2 diabetes, and an analogue is sometimes used in treating type 1 and type 2 DM. Human insulin is produced by recombinant DNA technology; structural alterations at one or more amino acid residues modify its physical and pharmacologic characteristics.

Secretion

Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters also influence insulin secretion. Glucose levels >3.9 mmol/L (70 mg/dL) stimulate insulin synthesis, primarily by enhancing protein translation and processing. Glucose stimulation of insulin secretion begins with its transport into the beta cell by a facilitative glucose transporter **(Fig. 3)**. Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibits the activity of an ATP-sensitive K+ channel. This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g., sulfonyl-ureas, meglitinides); the other is an inwardly

rectifying K+ channel protein (Kir6.2). Inhibition of this K+ channel induces beta cell membrane depolarization, which opens voltage-dependent calcium channels (leading to an influx of calcium), and stimulates insulin secretion. Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min, superimposed upon greater amplitude oscillations of about 80–150 min. Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose stimulated insulin secretion and suppress glucagon secretion. Glucagon-like peptide 1 (GLP-1), the most potent incretin, is released from L cells in the small intestine and stimulates insulin secretion only when the blood glucose is above the fasting level. Incretin analogues, are used to enhance endogenous insulin secretion.

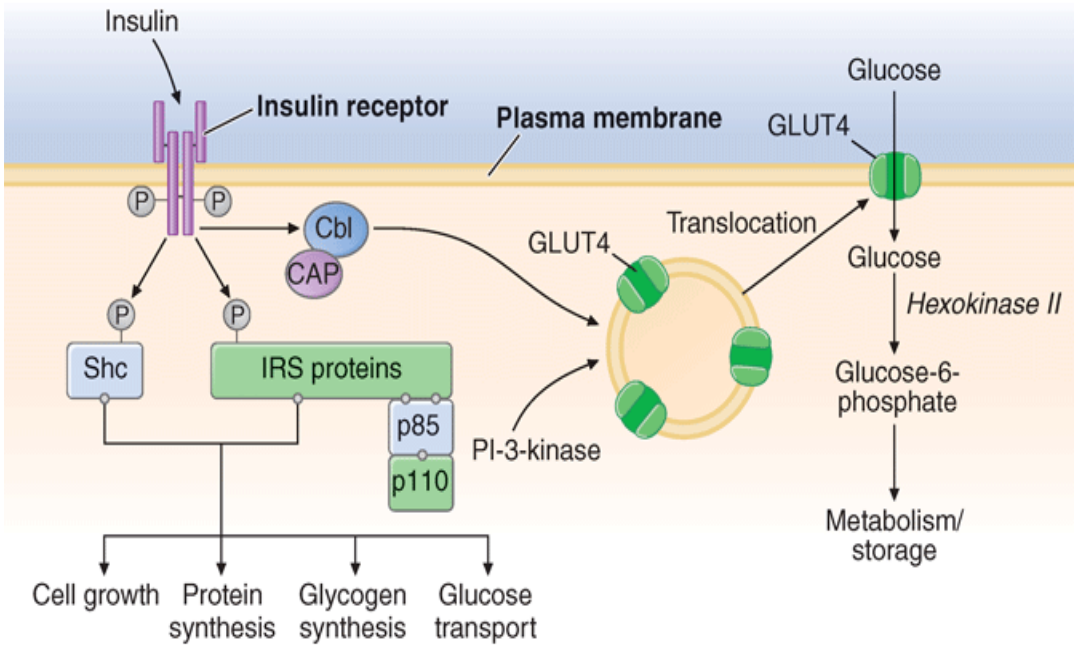


Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 3:- *Mechanisms of glucose-stimulated insulin secretion and abnormalities in diabetes*

Action

Once insulin is secreted into the portal venous system, 50% is removed and degraded by the liver. Unextracted insulin enters the systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the recruitment of intracellular signaling molecules, such as insulin receptor substrates (IRS) (Fig. 4). IRS and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin. As an example, activation of the phosphatidylinositol-3′-kinase (PI-3-kinase) pathway stimulates translocation of a facilitative glucose transporter (e.g., GLUT4) to the cell surface, an event that is crucial for glucose uptake by skeletal muscle and fat. Activation of other insulin receptor signaling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes in insulin-responsive cells.



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 4 *Insulin signal transduction pathway in skeletal muscle*

Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and other hormones (e.g., glucagon) result in integrated control of glucose supply and utilization. In the fasting state, low insulin levels increase glucose production by promoting hepatic gluconeogenesis and glycogenolysis and reduce glucose uptake in insulin-sensitive tissues (skeletal muscle and fat), thereby promoting mobilization of stored precursors such as amino acids and free fatty acids (lipolysis). Glucagon, secreted by pancreatic alpha cells when blood glucose or insulin levels are low, stimulates glycogenolysis and gluconeogenesis by the liver and renal medulla. Postprandially, the glucose load elicits a rise in insulin and fall in glucagon, leading to a reversal of these processes. Insulin, an anabolic hormone, promotes the storage of carbohydrate and fat and protein synthesis. The major portion of postprandial glucose is utilized by skeletal muscle, an effect of insulin-stimulated glucose uptake. Other tissues, most notably the brain, utilize glucose in an insulin-independent fashion. [21]

PATHOGENESIS AND PATHOPHYSIOLOGY OF DIABETES MELLITUS

There is a direct link between hyperglycemia and physiological & behavioral responses. Whenever there is hyperglycemia, the brain recognizes it and send a message through nerve impulses to pancreas and other organs to decrease its effect [22].

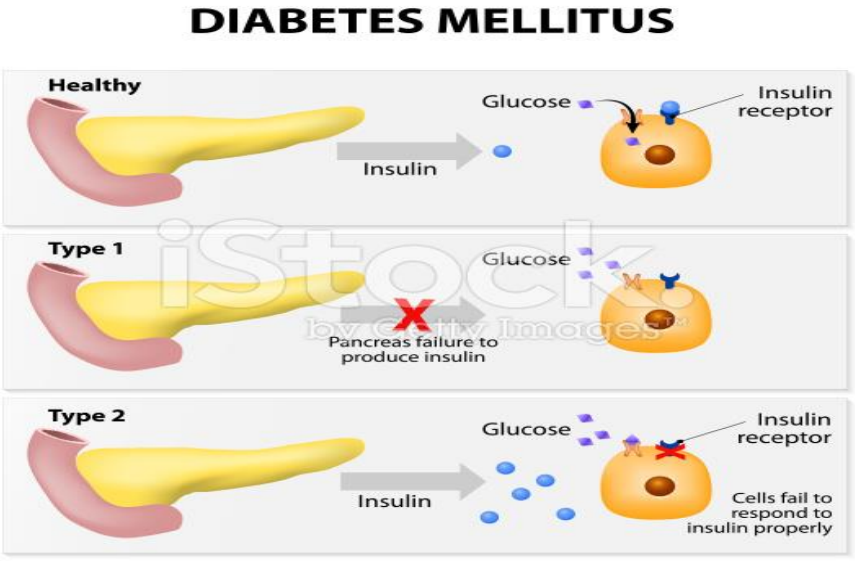


Figure 5:- *Normal and Type of Diabetes*

***PATHOGENESIS OF TYPE 1 DIABETES MELLITUS* (IDDM)**

Type 1 diabetes mellitus is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic β-cells (Figure 5) by CD4+ and CD8+ T cells and macrophages infiltrating the islets. The onset of clinical disease represents the end stage of β-cell destruction leading to type 1 diabetes mellitus. Several features characterize type 1 diabetes mellitus as an autoimmune disease [24, 25].

1. Presence of immuno-competent and accessory cells in infiltrated pancreatic islets;

2. Association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC; human leucocyte antigens HLA);

3. Presence of islet cell specific autoantibodies;

4. Alterations of T cell mediated immunoregulation, in particular in CD4+ T cell compartment;

5. The involvement of monokines and TH1 cells producing interleukins in the disease process;

6. Response to immunotherapy and;

7. Frequent occurrence of other organ specific auto- immune diseases in affected individuals Or in their family members.

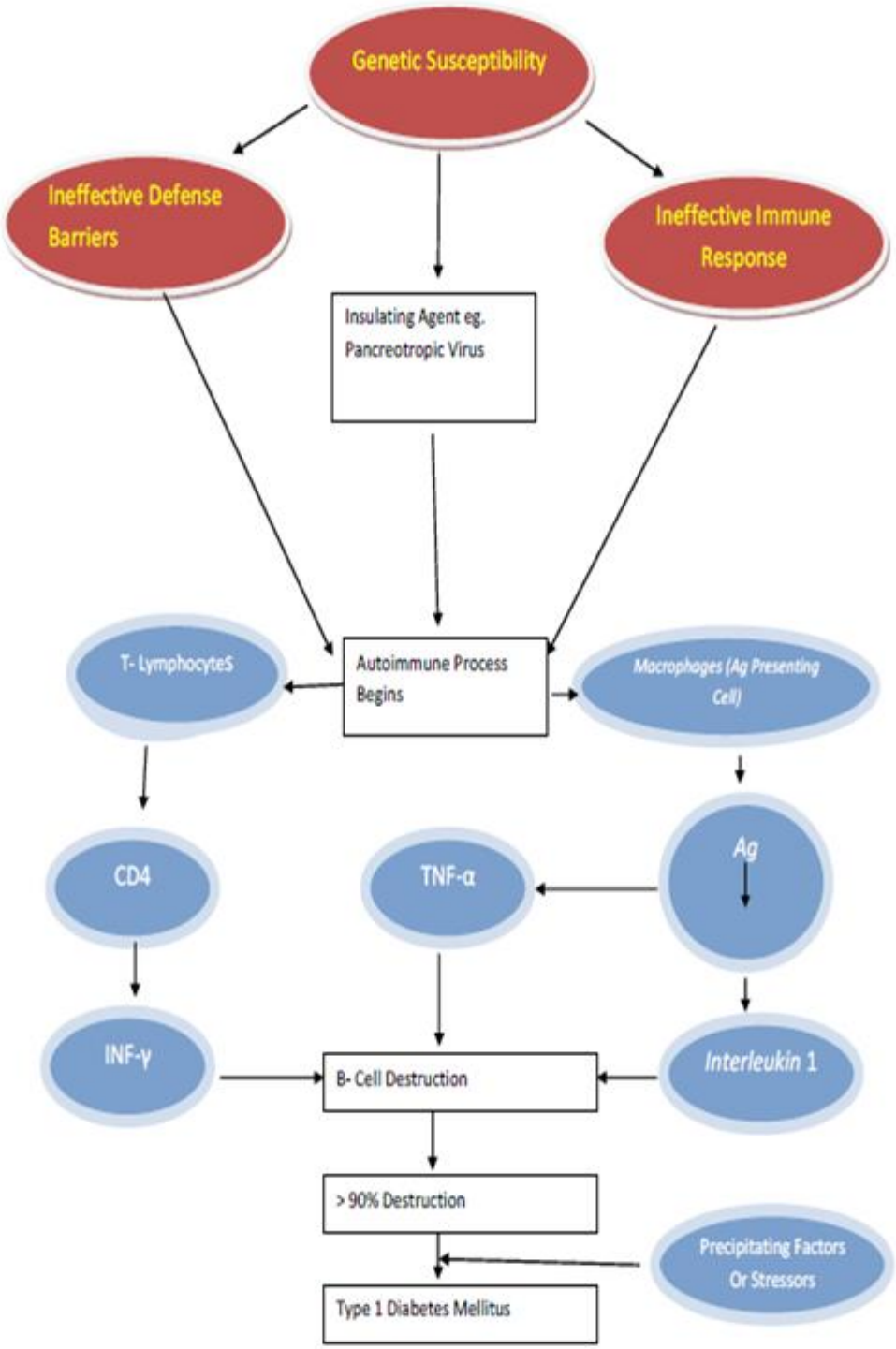


Figure 6:- *pathogenesis of type 1 diabetes mellitus*

PATHOPHYSIOLOGY TYPE 1 DIABETES MELLITUS (IDDM)

Approximately 85% of patients have circulating islet cell antibodies, and the majorities also have detectable anti-insulin antibodies before receiving insulin therapy. Most islet cell antibodies are directed against glutamic acid decarboxylase (GAD) within pancreatic B cells [26].

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with T1DM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagon in T1DM patients. Normally, hyperglycemia leads to reduced glucagon secretion, however, in patients with T1DM, glucagon secretion is not suppressed by hyperglycemia [26, 27]. The resultant inappropriately elevated glucagon levels exacerbate the metabolic defects due to insulin deficiency. Although insulin deficiency is the primary defect in T1DM, there is also a defect in the administration of insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle [27]. This impairs glucose utilization and insulin deficiency also decreases the expression of a number of genes necessary for target tissues to respond normally to insulin such as glucokinase in liver and the GLUT 4 class of glucose transporters in adipose tissue [34] explained that the major metabolic derangements, which result from insulin deficiency in T1DM are impaired glucose, lipid and protein metabolism. [13]

Effects on glucose metabolism

Uncontrolled IDDM leads to increased hepatic glucose output. First, liver glycogen stores are mobilized then hepatic gluconeogenesis is used to produce glucose. Insulin deficiency also impairs non hepatic tissue utilization of glucose. In particular in adipose tissue and skeletal muscle, insulin stimulates glucose uptake. This is accomplished by insulin mediated movement of glucose transporters proteins to the plasma membrane of these tissues. Reduced glucose uptake by peripheral tissues in turn leads to a reduced rate of glucose metabolism. In addition, the level of hepatic glucokinase is regulated by insulin. Therefore, a reduced rate of glucose phosphorylation in hepatocytes leads to increased delivery to the blood. Other enzymes involved in anabolic metabolic metabolism of glucose are affected by insulin.

The combination of increased hepatic glucose production and reduced peripheral tissues metabolism leads to elevated plasma glucose levels. When the capacity of the kidneys to absorb

glucose is suppressed, glucosuria ensues. Glucose is an osmotic diuretic and an increase in renal loss of glucose is accompanied by loss of water and electrolyte. The result of the loss of water (and overall volume) leads to the activation of the thirst mechanism (polydipsia). The negative caloric balance, which results from the glucosuria and tissue catabolism leads to an increase in appetite and food intake that is polyphagia [26].

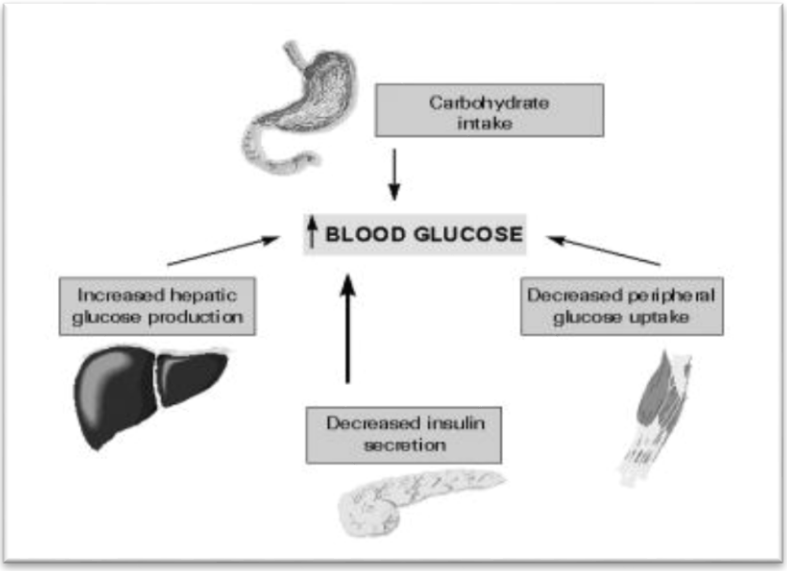


***Figure 7**:- glucose metabolism*

Effect on lipid metabolism

One major role of insulin is to stimulate the storage of food energy in the form of glycogen in hepatocytes and skeletal muscle, following the consumption of a meal. In addition, insulin stimulates hepatocytes to synthesize and store triglycerides in adipose tissue. In uncontrolled IDDM there is a rapid mobilization of triglycerides leading to increased levels of plasma free fatty acids. The free fatty acids are taken up by numerous tissue (except the brain) and metabolized to provide energy. In the absence of insulin, malonyl CoA levels fall, and transport of fatty acyl-CoA into the mitochondria increases. Mitochondrial oxidation of fatty acids generates acetyl CoA that can be further oxidized in the TCA cycle. However, in hepatocytes the majority of the acetyl CoA is not oxidized by the TCA cycle but is metabolized into the ketone bodies (acetoacetate and B-hydroxybutyrate). These ketone bodies are used for energy production by the brain, heart and skeletal muscle. In IDDM, the increased availability of free fatty acids and ketone bodies

exacerbates the reduced utilization of glucose, furthering the ensuing hyperglycemia. Production of ketone bodies in excess of the body's ability to utilize them leads to ketoacidosis. A spontaneous breakdown product of acetoacetate is the acetone that is exhaled by the lungs, which gives a distinctive odor to the breath. Normally, plasma triglycerides are acted upon by lipoprotein lipase (LPL) that requires insulin. LPL is a membrane bound enzyme on the surface of the endothelial cells lining the vessels, which allows fatty acids to be taken from circulating triglycerides for storage in adipocytes [26]. The absence of insulin results in hypertriglyceridemia.

Effects on protein

Insulin regulates the synthesis of many genes, either positively or negatively, which affect overall metabolism. Insulin has an overall effect on protein metabolism, increasing the rate of protein synthesis and decreasing the rate of protein degradation. Thus insulin deficiency will lead to increased catabolism of protein. The increased rate of proteolysis leads to elevated concentration of amino acids in plasma [26].Glucogenic amino acids serve as precursors for hepatic and renal glyconeogenesis, which further contributes to the hyperglycemia seen in IDDM

IMMUNOLOGIC MARKERS

Islet cell autoantibodies (ICAs) are a composite of several different antibodies directed at pancreatic islet molecules such as GAD, insulin, IA-2/ICA-512, and ZnT-8, and serve as a marker of the autoimmune process of type 1 DM. Assays for autoantibodies to GAD-65 are commercially available. Testing for ICAs can be useful in classifying the type of DM as type 1 and in identifying non-diabetic individuals at risk for developing type 1 DM. ICAs are present in the majority of individuals (>85%) diagnosed with new-onset type 1 DM, in a significant minority of individuals with newly diagnosed type 2 DM (5–10%), and occasionally in individuals with GDM (<5%). ICAs are present in 3–4% of first degree relatives of individuals with type 1 DM. In combination with impaired insulin secretion after IV glucose tolerance testing, they predict a >50% risk of developing type 1 DM within 5 years. At present, the measurement of ICAs in non-diabetic individuals is a research tool because no treatments have been approved to prevent the occurrence or progression to type 1 DM. Clinical trials are testing interventions to slow the autoimmune beta cell destruction.[28]

ENVIRONMENTAL FACTORS

Numerous environmental events have been proposed to trigger the autoimmune process in genetically susceptible individuals; however, none have been conclusively linked to diabetes. Identification of an environmental trigger has been difficult because the event may precede the onset of DM by several years. Putative environmental triggers include viruses (coxsackie, rubella, enteroviruses most prominently), bovine milk proteins, and nitrosourea compounds. [28]

PREVENTION OF TYPE 1 DM

A number of interventions have successfully delayed or prevented diabetes in animal models. Some interventions have targeted the immune system directly (immunosuppression, selective T-cell subset deletion, induction of immunologic tolerance to islet proteins), whereas others have prevented islet cell death by blocking cytotoxic cytokines or increasing islet resistance to the destructive process. Though results in animal models are promising, these interventions have not been successful in preventing type 1 DM in humans.

The Diabetes Prevention Trial--type 1 concluded that administering insulin (IV or PO) to individuals at high risk for developing type 1 DM did not prevent type 1 DM. In patients with new-onset type 1 diabetes, treatment with anti-CD3 monoclonal antibodies, a GAD vaccine, and anti-B lymphocyte monoclonal antibody have been shown to slow the decline in C-peptide levels. This is an area of active clinical investigation. [28]

Clinical Manifestations

- Polyuria : increased frequency of urination, and particularly urination at night (nocturia).
- Polydipsia : increased thirst.
- Polyphagia : increased hunger.
- Fatigue or lethargy.
- Weight loss.
- Recurrent infections like urinary tract infection or skin infection.
- Symptoms of ketoacidosis which include drowsiness, rapid breathing, dehydration, abdominal pain, nausea and vomiting, decreased consciousness or coma.[a]

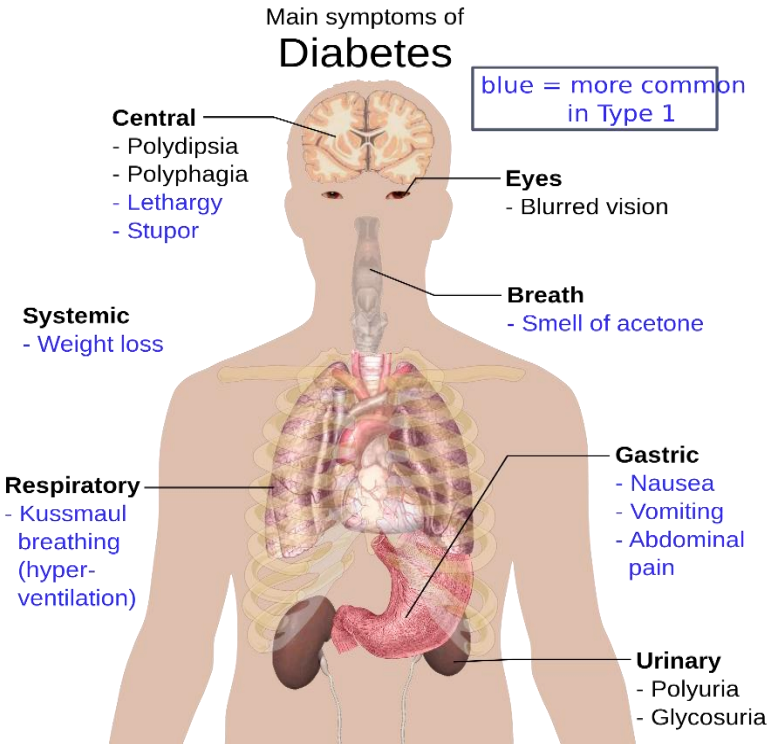


Figure 8:- *main symptoms of diabetes mellitus*

PATHOGENESIS TYPE 2 DIABETES MELLITUS (NIDDM)

Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in liver) and insulin secretion [29]. Two main pathological defects in type 2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance [30]. Type 2 diabetes mellitus has a greater genetic association than type 1 DM, the pathogenesis of type 2 diabetes mellitus is characterized by impaired insulin secretion and insulin resistance as shown in **Fig 9** [31].

Insulin resistance

The primary events are believed to be an initial deficit in insulin secretion and in many patients relative insulin deficiency in association with peripheral insulin resistance [34]. Resistance to the action of insulin will result in impaired insulin mediated glucose uptake in the periphery (by muscle and fat), incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance, islet cells will increase the amount of insulin secreted. Endogenous glucose production is accelerated in patients with type 2 diabetes or impaired fasting glucose. Because this increase occurs in the presence of hyper insulinemia, at least in the early and intermediate disease stages, hepatic insulin resistance is the driving force of hyperglycemia of type 2 diabetes

Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance [32]. Except for maturity onset diabetes of the young (MODY), the mode of inheritance for type 2 diabetes mellitus is unclear. MODY, inherited as an autosomal dominant trait, may result from mutations in glucokinase gene on chromosome 7p. MODY is defined as hyperglycemia diagnosed before the age of twenty-five years and treatable for over five years without insulin in cases where islet cell antibodies (ICA) are negative [33].

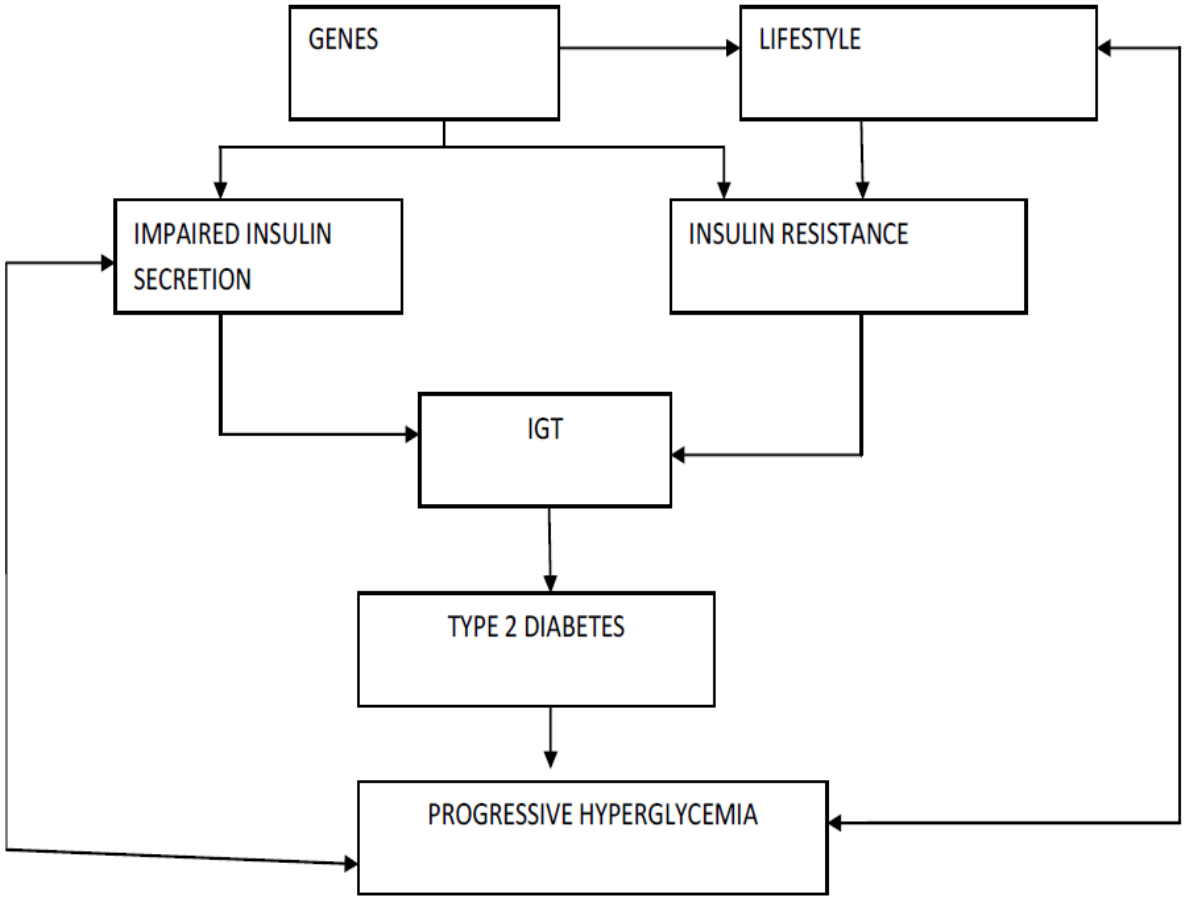


Figure 9:-*Pathogenesis of type 2 diabetes characterized by impaired insulin secretion and insulin resistance*

PATHOPHYSIOLOGY OF TYPE 2 DIABETES (NIDDM)

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM.the pathophysiology of type 2 diabetes is described in **Fig 10**. [35]

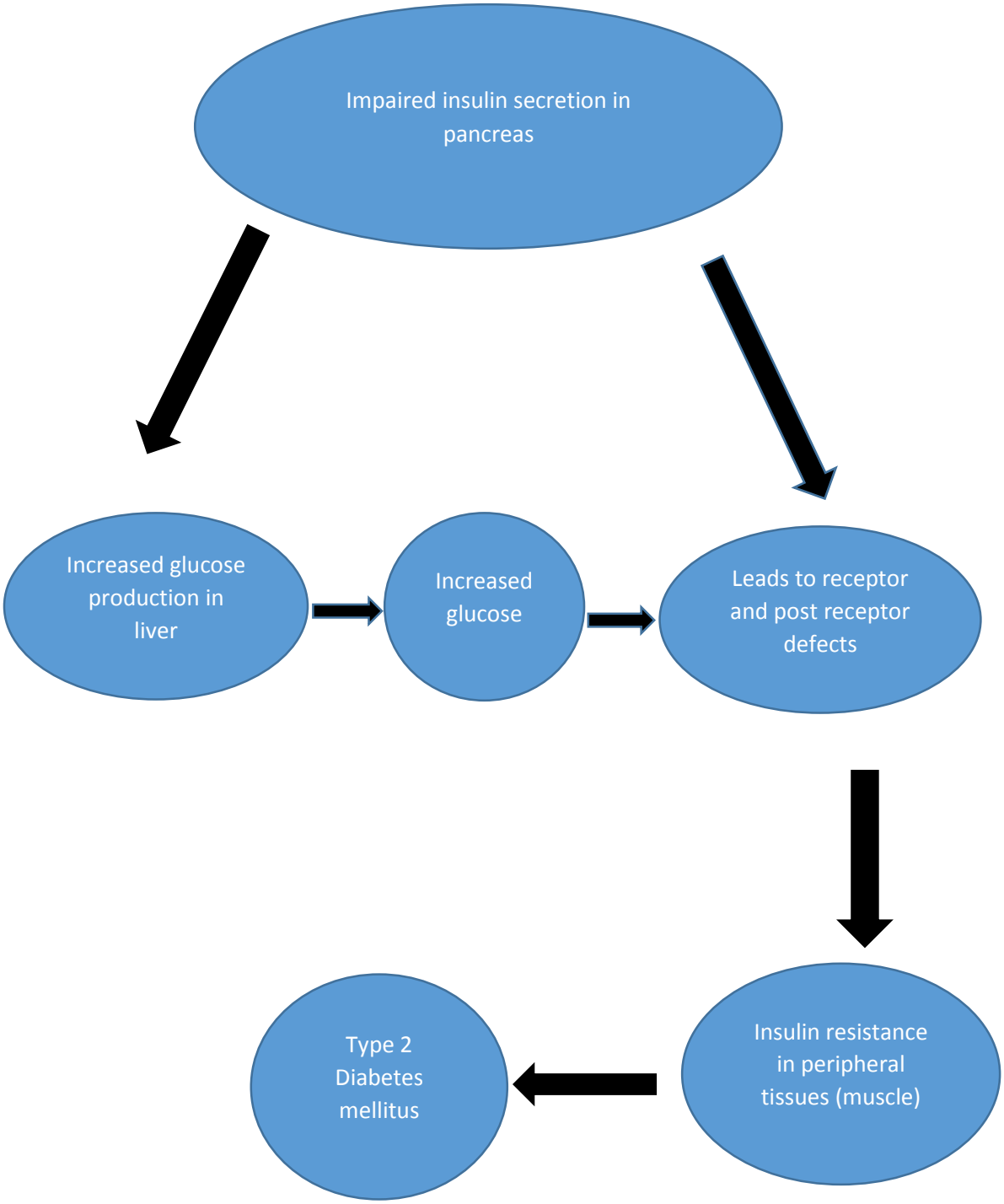
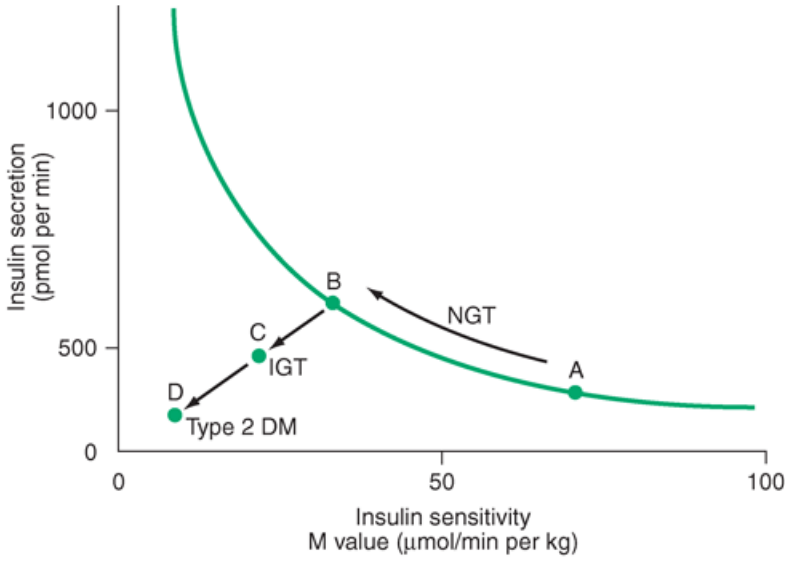


Figure 10:-*Pathophysiology of type 2 diabetes mellitus.*

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 DM (80% or more are obese). In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output **(Fig. 11)**. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state. Impaired Glucose Tolerance, characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues.



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Figure 11:-*Metabolic changes during the development of type 2 diabetes mellitus (DM)*

Insulin secretion and insulin sensitivity are related, and as an individual becomes more insulin resistant (by moving from point A to point B), insulin secretion increases. A failure to compensate by increasing the insulin secretion results initially in impaired glucose tolerance (IGT; point C) and ultimately in type 2 DM (point D). [36]

METABOLIC ABNORMALITIES

Abnormal Muscle and Fat Metabolism

Insulin resistance, the decreased ability of insulin to act effectively on target tissues (especially muscle, liver, and fat), is a prominent feature of type 2 DM and results from a combination of genetic susceptibility and obesity. Insulin resistance impairs glucose utilization by insulin-sensitive tissues and increases hepatic glucose output; both effects contribute to the hyperglycemia. Increased hepatic glucose output predominantly accounts for increased FPG levels, whereas decreased peripheral glucose usage results in postprandial hyperglycemia. In skeletal muscle, there is a greater impairment in non-oxidative glucose usage (glycogen formation) than in oxidative glucose metabolism through glycolysis. Glucose metabolism in insulin-independent tissues is not altered in type 2 DM. The precise molecular mechanism leading to insulin resistance in type 2 DM has not been elucidated. Insulin receptor levels and tyrosine kinase activity in skeletal muscle are reduced, but these alterations are most likely secondary to hyperinsulinemia and are not a primary defect. Therefore, "post receptor" defects in insulin-regulated phosphorylation/de-phosphorylation appear to play the predominant role in insulin resistance (**Fig.5**). For example, a PI-3-kinase signaling defect might reduce translocation of GLUT4 to the plasma membrane. Other abnormalities include the accumulation of lipid within skeletal myocytes, which may impair mitochondrial oxidative phosphorylation and reduce insulin-stimulated mitochondrial ATP production. Impaired fatty acid oxidation and lipid accumulation within skeletal myocytes also may generate reactive oxygen species such as lipid peroxides. Of note, not all insulin signal transduction pathways are resistant to the effects of insulin (e.g., those controlling cell growth and differentiation using the mitogenic-activated protein kinase pathway). Consequently, hyperinsulinemia may increase the insulin action through these pathways, potentially accelerating diabetes-related conditions such as atherosclerosis. The obesity accompanying type 2 DM, particularly in a central or visceral location, is thought to be part of the pathogenic process. The increased adipocyte mass leads to increased levels of circulating free fatty acids and other fat cell products. The increased production of free fatty acids and some adipokines may cause insulin resistance in skeletal muscle and liver. For example, free fatty acids impair glucose utilization in skeletal muscle, promote glucose production by the liver, and impair beta cell function. In contrast,

the production by adipocytes of adiponectin, an insulin-sensitizing peptide, is reduced in obesity, and this may contribute to hepatic insulin resistance. Adipocyte products and adipokines also produce an inflammatory state and may explain why markers of inflammation such as IL-6 and C-reactive protein are often elevated in type 2 DM. In addition, inflammatory cells have been found infiltrating adipose tissue. Inhibition of inflammatory signaling pathways such as the nuclear factor B (NF- B) pathway appears to reduce insulin resistance and improve hyperglycemia in animal models. [28]

IMPAIRED INSULIN SECRETION

Insulin secretion and sensitivity are interrelated (**Fig. 11**). In type 2 DM, insulin secretion initially increases in response to insulin resistance to maintain normal glucose tolerance. Initially, the insulin secretory defect is mild and selectively involves glucose-stimulated insulin secretion. The response to other non-glucose secretagogues, such as arginine, is preserved. Abnormalities in proinsulin processing is reflected by increased secretion of proinsulin in type 2 diabetes. Eventually, the insulin secretory defect progresses to a state of inadequate insulin secretion. The reason(s) for the decline in insulin secretory capacity in type 2 DM is unclear. The assumption is that a second genetic defect—superimposed upon insulin resistance—leads to beta cell failure. Beta cell mass is decreased by approximately 50% in individuals with long-standing type 2 diabetes. Islet amyloid polypeptide or amylin is co-secreted by the beta cell and forms the amyloid fibrillar deposit found in the islets of individuals with long-standing type 2 DM. Whether such islet amyloid deposits are a primary or secondary event is not known. The metabolic environment of diabetes may also negatively impact islet function. For example, chronic hyperglycemia paradoxically impairs islet function ("glucose toxicity") and leads to a worsening of hyperglycemia. Improvement in glycemic control is often associated with improved islet function. In addition, elevation of free fatty acid levels ("lipotoxicity") and dietary fat may also worsen islet function. [28]

INCREASED HEPATIC GLUCOSE AND LIPID PRODUCTION

In type 2 DM, insulin resistance in the liver reflects the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycemia and decreased glycogen storage by the liver in the postprandial state. Increased hepatic glucose production occurs early in the course of

diabetes, though likely after the onset of insulin secretory abnormalities and insulin resistance in skeletal muscle. As a result of insulin resistance in adipose tissue, lipolysis and free fatty acid flux from adipocytes are increased, leading to increased lipid [very low density lipoprotein (VLDL) and triglyceride] synthesis in hepatocytes. This lipid storage or steatosis in the liver may lead to nonalcoholic fatty liver disease and abnormal liver function tests. This is also responsible for the dyslipidemia found in type 2 DM [elevated triglycerides, reduced high-density lipoprotein (HDL), and increased small dense low-density lipoprotein (LDL) particles].[28]

INSULIN RESISTANCE SYNDROMES

The insulin resistance condition comprises a spectrum of disorders, with hyperglycemia representing one of the most readily diagnosed features. The *metabolic syndrome*, the *insulin resistance syndrome*, or *syndrome X* are terms used to describe a constellation of metabolic derangements that includes insulin resistance, hypertension, dyslipidemia (decreased HDL and elevated triglycerides), central or visceral obesity, type 2 diabetes or IGT/IFG, and accelerated cardiovascular disease.

A number of relatively rare forms of severe insulin resistance include features of type 2 DM or IGT (**Table 2**). Mutations in the insulin receptor that interfere with binding or signal transduction are a rare cause of insulin resistance. Acanthosis nigricans and signs of hyperandrogenism (hirsutism, acne, and oligomenorrhea in women) are also common physical features.

Two distinct syndromes of severe insulin resistance have been described in adults:

- Type A, which affects young women and is characterized by severe hyperinsulinemia, obesity, and features of hyperandrogenism; and
- Type B, which affects middle-aged women and is characterized by severe hyperinsulinemia, features of hyperandrogenism, and autoimmune disorders.

Individuals with the type A insulin resistance syndrome have an undefined defect in the insulin-signaling pathway; individuals with the type B insulin resistance syndrome have autoantibodies directed at the insulin receptor. These receptor autoantibodies may block insulin binding or may stimulate the insulin receptor, leading to intermittent hypoglycemia. Polycystic ovary syndrome (PCOS) is a common disorder that affects premenopausal women and is characterized by chronic

anovulation and hyperandrogenism. Insulin resistance is seen in a significant subset of women with PCOS, and the disorder substantially increases the risk for type 2 DM, independent of the effects of obesity. [28]

RISK FACTORS: [37]

- Obesity
- Age (onset of puberty is associated with increased insulin resistance)
- Lack of physical activity
- Genetic predisposition
- Racial/ethnic background (African American, Native American, Hispanic and Asian/Pacific Islander)
- Conditions associated with insulin resistance, (e.g., polycystic ovary syndrome).
- Family history of T2DM in first- or second-degree relative[38,13]
- High-calorie diet

COMPLICATIONS OF DIABETES MELLITUS [13]

I. Acute complications

- Hypoglycemia
- Hyperglycemic crises
    a. Diabetes Ketoacidosis (DKA)
    b. Hyperglycemic hyperosmolar state (HHS)

II. Chronic complications:

- Micro vascular complications
    a. Diabetic retinopathy
    b. Diabetic nephropathy
    c. Diabetic neuropathy
- Macro vascular disease

3 Other complications and associated conditions

- Impaired growth and development

- Associated autoimmune conditions
    a. Hypothyroidism
    b. Hyperthyroidism
    c. Celiac disease
    d. Vitiligo
    e. Primary adrenal insufficiency (Addison's disease)
- Lipodystrophy (lipoatrophy and lipohypertrophy)
- Necrobiosis lipoidica diabeticorum
- Non-alcoholic fatty liver disease
- Infections seen in patients with diabetes
- Limited joint mobility
- Edema

Diabetes Ketoacidosis (DKA)

DKA results from relative or absolute insulin deficiency combined with counter regulatory hormone excess (glucagon, catecholamine, cortisol, and growth hormone). Both insulin deficiency and glucagon excess, in particular, are necessary for DKA to develop. The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis, and ketone body formation in the liver, as well as increases in substrate delivery from fat and muscle (free fatty acids, amino acids) to the liver.

Nausea and vomiting, abdominal pain which resemble acute pancreatitis or ruptured viscus, Kussmaul respirations and a fruity odor on the patient's breath (secondary to metabolic acidosis and increased acetone) are classic signs of the disorder. [39]

Hyperglycemic hyperosmolar state (HHS)

The prototypical patient with HHS is an elderly individual with type 2 DM, with a several-week history of polyuria, weight loss, and diminished oral intake that culminates in mental confusion, lethargy, or coma. The physical examination reflects profound dehydration and hyper osmolality and reveals hypotension, tachycardia, and altered mental status. Notably absent are symptoms of nausea, vomiting, and abdominal pain and the Kussmaul respirations characteristic of DKA. [39]

Chronic Complications

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications.

The vascular complications of DM are further subdivided into micro vascular (retinopathy, neuropathy, and nephropathy) and macro vascular complications [coronary heart disease (CHD), peripheral arterial disease (PAD), cerebrovascular disease].

Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be associated with hearing loss. [40]

Diabetic retinopathy

DM is the leading cause of blindness. Blindness is primarily the result of progressive diabetic retinopathy and clinically significant macular edema. Diabetic retinopathy is classified into two stages: non proliferative and proliferative.

Non proliferative diabetic retinopathy usually appears late in the first decade or early in the second decade of the disease and is marked by retinal vascular micro aneurysms, blot hemorrhages, and cotton-wool spots. Mild non proliferative retinopathy progresses to more extensive disease, characterized by changes in venous vessel caliber, intraretinal micro vascular abnormalities, and more numerous microaneurysms and hemorrhages. [40]

Necrobiosis lipoidica diabeticorum

It is a rare disorder of DM that predominantly affects young women with type 1 DM, neuropathy, and retinopathy. It usually begins in the pretibial region as an erythematous plaque or papules that gradually enlarge, darken, and develop irregular margins, with atrophic centers and central ulceration. They may be painful. [40]

Lipoatrophy and lipohypertrophy

It occur at insulin injection sites but are now unusual with the use of human insulin. Xerosis and pruritus are common and are relieved by skin moisturizers. [40]

DIAGNOSIS OF DIABETES MELLITUS

Random plasma test

• The simplest test and doesn't require fasting before taking the test.

• If 200 or more than 200 mg/dl of blood glucose it probably indicates diabetes but has to be reconfirmed.

Fasting plasma glucose test:

• There should be eight hours fasting before taking this test. Blood glucose more than 126 mg/dl on two or more tests conducted on different days confirms a diabetes diagnosis [41].

Oral glucose tolerance test

• When random plasma glucose test is 160-200 mg/dl and the fasting plasma test is 110-125 mg/dl, then this test is conducted [42].

• This blood test evaluates body's response to glucose. This test requires fasting at least eight but not more than 16 hrs.

• Fasting glucose level is determined, and then gives 75 gm of glucose, 100 gm for pregnant women. The blood is tested every 30 minutes to one hr for two or three hrs.

• This test is normal if your glucose level at two hrs is less than 140 mg/dl. A fasting level of 126 mg/dl or greater and two hour glucose level of 200 mg/dl or higher confirms a diabetes diagnosis [41].

MANAGEMENT OF DIABETICS MELLITUS

Treatment for Type 1 Diabetes

Insulin Therapy

Insulin is lifesaving pharmacological therapy for people with type 1 diabetes. Insulin preparations are primarily produced by recombinant DNA technology and are formulated either as structurally identical to human insulin or as a modification of human insulin (insulin analogues) to alter

pharmacokinetics. Human insulin and insulin analogues are preferred and used by most adults with type 1 diabetes [43]

Insulin is the only medication that is effective in lowering blood glucose levels in type 1 diabetes. The use of insulin requires daily management of those factors that affect the insulin dose (food, physical activity, illness, stress). Rapid-acting insulin may be given before, during, or immediately after a meal. Administration after a meal may help reduce the postprandial hyperglycemia associated with high fat meals. The number of insulin injections/day will vary; insulin may be delivered with insulin syringes, insulin pens or external insulin pumps. [37]

Insulin preparations are classified according to their duration of action and are further differentiated by their time of onset and peak actions. Premixed insulin preparations are available and are not generally suitable for intensive treatment in patients with type 1 diabetes in whom frequent adjustments of insulin are required. [43]

Types of Insulin [44]

Insulin Preparation	Name	Onset	Peak	Duration
Rapid-acting insulin Analogues (Clear)	**Insulin aspart** **Insulin Lispro** **Insulin Glulisine**	5 to 15 minutes	1 to 2 hours	4 to 5 hours
Short-acting Insulin (Clear)	**Humulin-Regular Or regular insulin**	20 to 60 minutes	2 to 4 hours	8 to 10 hours
Intermediate acting (Cloudy)	**Humulin-NPH or NPH**	1 to 2 hours	4 to 8 hours	10 to 20 hours

Long-acting Insulin analogues (Clear)	**Insulin detemir** **Insulin glargine**	1 to 2 hours 1 to 2 hours	Relatively flat Relatively flat	12 to 20 hours 20 to 24 hours
Pre-Mixed insulin Multiple preparations	**NPH/Regular mix** PreMix 70/30 (NPH/Regular) (Premix ***Regular)*** **NPH/Rapid Acting Insulin mix** **NPH/ Aspart** PreMix Novolog 70/30 **NPH/Lispro** PreMix Humalog 75/25 (Premix ***Rapid acting)***		A single vial or cartridge contains a fixed ratio of insulin (% of rapid-acting or short-acting insulin to % of intermediate- acting insulin)	

Table 4:- *Insulin Preparations*

Treatment for Type 2 Diabetes

General Aspects

The goals of therapy for type 2 DM are similar to those in type 1. While glycemic control tends to dominate the management type 2 DM must also include attention to the treatment of conditions associated with type 2 DM (obesity, hypertension, detection/management of DM-related

complications. DM-specific complications may be present in up to type 2 DM. Reduction in cardiovascular risk is of paramount importance as this is the leading cause of mortality in these and lipid goals should begin in concert with glucose-lowering interventions.[45]

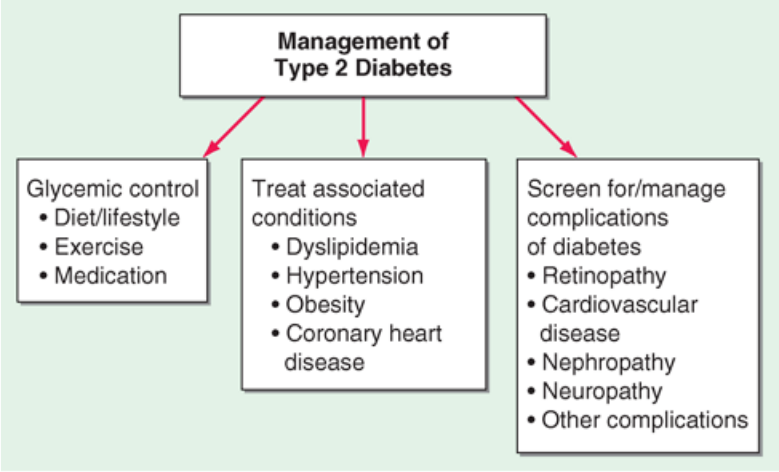


Figure 12:- *Management of Type 2 Diabetes*

CLASSES OF ORAL HYPOGLYCEMIC AGENTS[b]

Drug class	**Agent**
• Sulfonylureas	First generation
	Acetohexamide **Chlorpropamide** **Tolazamide** **Tolbutamide**
	Second generation
	Glyburide **Glipizide** **Glimepiride**
• Meglitinides	**Repaglinide** **Nateglinide**

• Biguanides	**Metformin**
• Thiazolidinediones	**Pioglitazone** **Rosiglitazone**
• Alpha-glucosidase inhibitors	**Acarbose** **Miglitol**
• GLP-1 agonist	**Exenatide** **Liraglutide**
• Dipeptidyl Peptidase-4 Inhibitors	**Saxagliptin** **Sitagliptin** **Vildagliptin**[45]

Table:-5 *oral hypoglycemic agents*

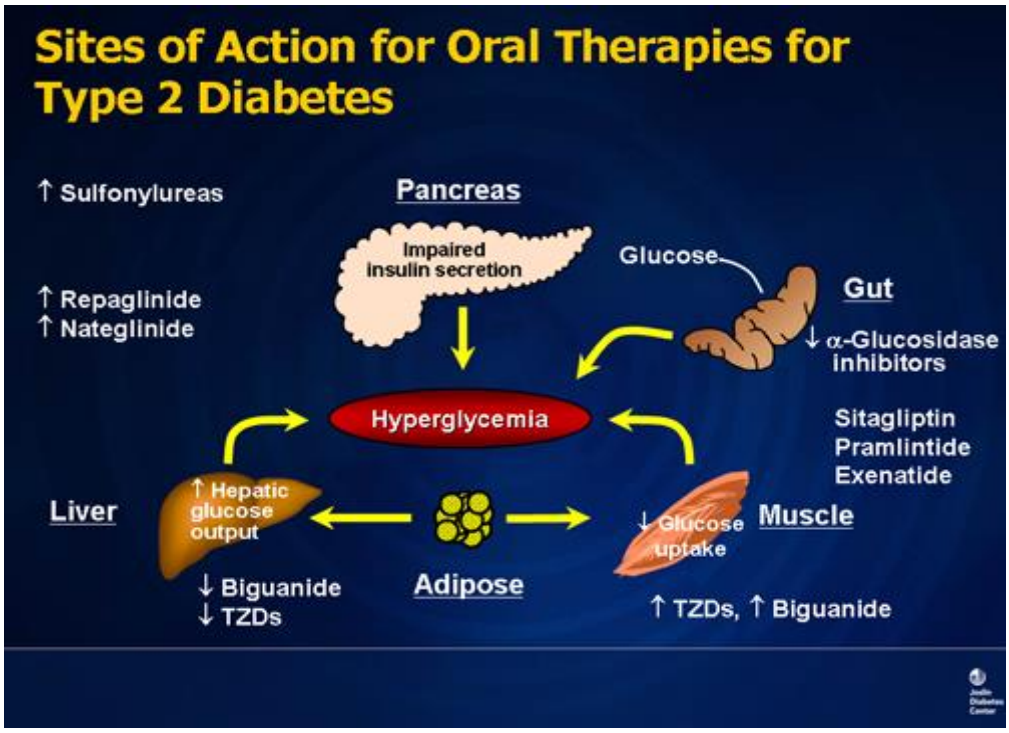


Figure:-13 *site of action of diabetic drugs*

MODELS FOR EXPERIMENTAL DIABETES MELLITUS

The various animal models for inducing diabetes are:

I. Chemical induction of diabetes mellitus:
    a. Streptozotocin induced diabetes
    b. Alloxan induced diabetes
    c. Ferric nitrilotriacetate induction of diabetes mellitus
    d. Other diabetogenic compounds
II. Surgical model of diabetes mellitus
    a. Pancreatectomy in dogs
III. Genetic models of diabetes
    a. Spontaneously develop diabetic rats
    b. Genetically engineered diabetic mice
IV. Hormone induced diabetes
V. Insulin deficiency due to insulin antibodies
VI. Virus induced diabetes [77,78]

Streptozotocin Model of Diabetes Mellitus

Streptozotocin or streptozocin or Izostazin or zanosar (STZ) is a synthetic nitrosoureido glucopyranose derivative isolated from fermentations of *Streptomyces achromogenes* that is classically an anti tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. The chemical name of Streptozotocin is 2-Deoxy-2[(methylnitrosoamino)-carbonyl] amino]-Dglucopyranose [78].It is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties. However, STZ is not a drug of choice for treatment of cancers due to development of resistance to its genotoxic effects. More specifically, STZ exhibits pancreatic beta-cell toxicity and is often used to induce diabetes in experimental animals [80]

How STZ Works

- Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid(DNA)
- The exact mechanism of targeted beta-cell toxicity is, however, not clear. STZ induces beta-cell dysfunction and apoptosis at lower doses while causing beta-cell necrosis at higher doses
- Insulin-secretory cells also develop resistance on repeated exposures to STZ through a wide spectrum of toxin tolerance mechanisms
- STZ induces oxidative stress by activation of poly adenosine diphosphate ribosylation and nitric oxide release.
- As a result of STZ action, pancreatic β-cells are destroyed by necrosis[79,80]

Single dose of STZ in sterile citrate buffer (e.g. pH 4.5 0.1M) may be used: mice 150 mg/kg; rats 80 mg/kg, administered intraperitoneally [78].After the administration of streptozotocin, three phases of blood glucose changes are observed. Initially, blood glucose is increased, reaching values of 150–200 mg%. Then the serum insulin values are increased up to 4 times, resulting in a hypoglycemic phase which is followed by persistent hyperglycemia. Severity and onset of diabetic symptoms depend on the dose of streptozotocin. After the dose of 60 mg/kg i.v, symptoms occur already after 24–48 hours with hyperglycemia up to 800 mg%, glucosuria and ketonemia. Histologically, the beta-cells are degranulated or even necrotic. After that steady state is reached allowing the use the animals for pharmacological tests. [77]

PROBLEMS ASSOCIATED WITH MODERN DRUG THERAPY [46, 47, 48, 49, 50]

INSULIN THERAPY

1. Hypoglycemia: Manifested by:-

   Coma due to blood glucose to the brain.

   Sympathetic: tachycardia, sweating, anxiety.

   Parasympathetic: Nausea, Vomiting
2. Weight gain

3. Hypersensitivity reactions.
4. Lipodystrophy at injection site
5. Insulin resistance
6. Hypokalemia

SULFONYLUREAS

1. Hyperinsulinemia & Hypoglycemia:

   More in chlorpropamide & glibenclamide

   Less in tolbutamide.

   More in elderly and patients with renal disease.
2. Weight gain due to increase in appetite
3. GIT upset.
4. Dilutional hyponatremia, water intoxication (Chlorpropamide) vasopressin effect.
5. Disulfiram-like reaction with alcohol (chlorpropamide).
6. Tachyphylaxis (secondary failure).

Contraindications

1. Pregnancy (use insulin)
2. Hepatic or renal insufficiency
3. Type I diabetes

MEGLITINIDES

1. less incidence than sulfonylureas
2. Hypoglycemia (meal is delayed).
3. Weight gain.
4. Drug interactions.

Contraindications

1. Hepatic and renal impairment.

BIGUANIDES

1. Metallic taste in the mouth
2. GIT disturbances (nausea, vomiting, diarrhea).
3. Lactic acidosis: Common in patients with renal disease, liver, pulmonary or cardiac disease.
4. Long term use interferes with B12absorption.

Contraindications

1. Pregnancy
2. Renal disease.
3. Liver disease.
4. Alcoholism.
5. Conditions predisposing to hypoxia as cardiopulmonary dysfunction.

THIAZOLIDINEDIONES

1. Fluid retention (Edema).
2. Weight gain.
3. Headache.
4. Liver function tests for 1styear of therapy.
5. Failure of estrogen-containing oral contraceptives

Contraindications

1. Heart failure.
2. Pregnancy.
3. Significant liver disease.

α-GLUCOSIDASE INHIBITORS

1. GIT: Flatulence, diarrhea, abdominal pain, bloating, increase in liver enzymes

Contraindications

1. Inflammatory bowel disorders (IBD).
2. Renal disease.

3. Hepatic disease (used with caution).
4. Intestinal obstruction

DIPEPTIDYL PEPTIDASE-4 INHIBITOR (DPP-4 INHIBITORS)

1. Nausea ,abdominal pain ,diarrhea
2. Nasopharyngitis[46,47,48,49,50]

ADVANTAGE OF THE USE OF HERBAL MEDICINE IN DIABETICS MELLITUS

Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Herbal marketers globally increase due to safe drug delivery with fewer side effect compared to synthetic drugs.

In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. In many journals, national and international, we find an increasing number of research publications based on herbal drugs. Many analysis-based studies regarding pharmacological research in India have been conducted in the past [53, 54, 55].

HERBAL DRUGS

"Herbal formulation mean a dosage form consisting of one or more herbs or processed herb(s) in specified quantities to provide specific nutritional, cosmetic benefits, and/or other benefits meant for use to diagnose treat, mitigate diseases of human beings or animals and/or to alter the structure or physiology of human beings or animals". Herbal preparations are obtained by subjecting whole plant, fragmented or cut plants, plants parts to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates [51].

SCOPE OF STUDY

As per ancient literature, more than 800 plants are reported to have antidiabetic properties [56]. Ethno pharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity [57]. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in ancient India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. Its earliest reference (1000 BC in the Ayurvedic literature) is found in mythological form where it is said to have originated by eating Havisha, [58] a special food, which used to be offered at the times of yagna organized by Dakshaprajapati.

Ayurvedic antidiabetic herbs improve digestive power, increase one of the Rasas (gastric secretions); being Laghu, get easily digested in the body; and being Ruksha, decrease output of overall body fluids e.g. urine, sweat etc. Food items, which are 'madhumehaghna' (antidote), are an important underlying principle of therapy for the prameha (diabetes) patient. Food items which correct the metabolic imbalance by their action e.g. foods exhibiting 'rasa', 'katu', 'laghu', 'medaghna', properties are old cereals, roasted cereals, barley, jawar, ragi, mung dal, horse gram, tur dal, drumstick leaves, bitter gourd, jamun, amla, fig, raw papaya, milk, meat of animals that live in dry region, etc. The indigenous diet may not be useful in lowering the blood sugar to the same extent as insulin and other hypoglycemic agents do. But it has some other influences, which may be useful for the management of the disease and its complications [59]. Indian materia medica has mentioned numerous dravyas, which have been reported effective in Madhumeha [60].

Plants-based products have been popular all over the world for the centuries. In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complications of the disease. Some herbs have also been proven to help in the regeneration of ß-cells and in overcoming resistance. In addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and cholesterol lowering action. The management of type 2 diabetes mellitus (NIDDM) is possible with the drugs that can lower the blood sugar level in one hand and restore the liver glycogen level on the other. In modern system of medicine, there is no drug, which is reported to possess both of these properties [61]. However, the hypoglycemic effect of some herbal extracts have been confirmed in human and animal models of type 2 diabetes and conventional drugs have been derived from the active

molecules of these medicinal plants. Metformin, a less toxic biguanides and potent oral glucose-lowering agent, was developed from *Galega officianalis* and used to treat diabetes [62]. Out of dozens of oral medications for diabetes, only one medication (metformin) is approved for use in children and it has been originated from herbs [63].

Mechanism of Action of Herbal Anti-diabetics [64, 65, 66]

The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal anti-diabetic may be

a. α –amylase inhibition.
b. Inhibition in renal glucose reabsorption.
c. Stimulation of insulin secretion from beta cells of islets or/and inhibition of insulin degradative processes.
d. Cortisol lowering activities.
e. Insulin resistance reduction.
f. Providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the β -cells.
g. Regenerating and/or repairing pancreatic β cells.
h. Increasing the size and number of cells in the islets of Langerhans.
i. Stimulation of insulin secretion.
j. Stimulation of glycogenesis and hepatic glycolysis.
k. Inhibition of β -galactocidase and α–glucocidase.
l. Protective effect on the destruction of the β cells.
m. Improvement in digestion along with reduction in blood sugar and urea.
n. Prevention of pathological conversion of starch to glucose.

Advantages [52]

1. Mostly herbal drugs are well tolerated by the patient, having fewer unintended consequences and fewer side effects than traditional medicine, and may be safer to use.

2. Herbal drugs are more effective for long-standing health complaints that don't respond well to traditional medicine.
3. Cost of herbal drugs is much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.
4. Herbs are available without a prescription. Simple herbs, such as peppermint and chamomile, can be cultivated at home.

Limitations [52]

1. An herbalist would not be able to treat serious trauma, such as a broken leg, nor would he be able to heal appendicitis or a heart attack as effectively as a conventional doctor using modern diagnostic tests, surgery, and drugs.
2. Self treatment with herbal drugs may consist of many risk factors. Moreover with no proper direction of doses may lead to overdose.
3. Consumption of herbal drugs without correct identification of plant i.e. use of wrong part of plant may lead to poisoning.
4. All herbal drugs are not safe; some may be poisonous or may cause allergenic reactions.
5. Curing period is usually longer in comparison to conventional medication. Immense patience while undergoing herbal treatment is needed.

SOME DRUGS USED FOR DIABETICS MELLITUS

Some herbs that have been confirmed by scientific investigation, which appear to be most effective, relatively non-toxic and have substantial documentation of efficiency. [67, c]

Cinnamon: It has insulin-like properties, which able to decrease blood glucose levels as well as triglycerides and cholesterol, all of which are important especially for type 2 diabetes patients.

Bitter melon (*Momordica charantia*): Studies suggested that Asian Bitter Melon may lower blood glucose concentrations. Several compounds have been isolated from bitter melon that is believed to be responsible for its blood-sugar-lowering properties. These include charantin and an

insulin-like protein referred to as polypeptide-P, or plant insulin. It is believed that bitter melon acts on both the pancreas and in non pancreatic cells, such as muscle cells.

Gynema Sylvestre: To treat diabetes, dried leaves are powdered together with Coriander fruit juice is extracted and given orally. These remedy has been used in India for treating diabetes for about 2000 years. Today in India it is being used to treat primarily type II diabetes and type I as well. Gymnema also improves the ability of insulin to lower blood sugar in both type I and type II diabetes. This herb is showing up in more and more over the counter weight loss products and blood sugar balancing formulas.

Onion: Onion is a member of the lily family (Liliaceae). Experimental and clinical evidence suggests that onion consists of an active ingredient called APDS (allyl propyl disulphide). APDS has been shown to block the breakdown of insulin by the liver and possibly to stimulate insulin production by the pancreas, thus increasing the amount of insulin and reducing sugar levels in the blood. The additional benefit of the use of garlic is it beneficial cardiovascular effects. It is found to lower lipid levels, inhibit platelet aggregation and are antihypertensive. So the use of onion is recommended for diabetes patients.

Fenugreek (Trigonella foenum-graecum): Fenugreek is used both as an herb (the leaves) and as a spice (the seed). Pre-clinical and clinical studies have demonstrated the antidiabetic properties of fenugreek seeds. The fiber-rich fraction of fenugreek seeds can lower blood sugar levels in people with diabetes, and to a lesser extent, for lowering blood cholesterol. Additionally, the soluble fiber content of fenugreek may play a role in aiding weight control.

Asian Ginseng: Asian ginseng is commonly used in traditional Chinese medicine to treat diabetes. It has been shown to enhance the release of insulin from the pancreas and to increase the number of insulin receptors. It also has a direct blood sugar-lowering effect and improves psycho-physiological performance.

Banaba (Lagerstroemia speciosa): Banaba possesses the powerful compound corosolic acid and tannins, including lagerstroemin that lends itself to the treatment of diabetes. These ingredients are

thought to stimulate glucose uptake and have insulin-like activity. The latter activity is thought to be secondary to activation of the insulin receptor tyrosine kinase or the inhibition of tyrosine phosphatase. It is a natural plant insulin, can be taken orally.

Babhul (Acacia arabica): It is found all over India mainly in the wild habitat. The plant extract acts as an antidiabetic agent by acting as secretagouge to release insulin.

Garlic (Allium sativum): Allicin, a sulfur-containing compound is responsible for its pungent odour and it has been shown to have significant hypoglycemic activity. This effect is thought to be due to increased hepatic metabolism, increased insulin release from pancreatic beta cells and/or insulin sparing effect, thus decreased fasting blood glucose, and triglyceride levels in serum in comparison to sucrose controls. S-allyl cystein sulfoxide (SACS), the precursor of allicin and garlic oil, is a sulfur containing amino acid, which controlled lipid peroxidation better than glibenclamide and insulin. It also improved diabetic conditions. SACS also stimulated *In-vitro* insulin secretion from beta cells isolated from normal rats

Aloe (Aloe vera and Aloe barbadensis): Extracts of aloe gum effectively increases glucose tolerance in both normal and diabetic rats. Treatment of chronic but no single dose of exudates of *Aloe barbadensis* leaves showed hypoglycemic effect. Single as well as chronic doses of bitter principle of the same plant also showed hypoglycemic effect. This action of *Aloe vera* and its bitter principle is through stimulation of synthesis and/or release of insulin from pancreatic beta cells. This plant also has an anti-inflammatory activity in a dose dependent manner and improves wound healing in diabetes.

Neem (Azadirachta indica): Hydroalcoholic extracts of this plant showed anti-hyperglycemic activity. Apart from having anti-diabetic activity, this plant also has anti-bacterial, antimalarial, antifertility, hepatoprotective and antioxidant effects.

Mango (Mangifera indica): The leaves of this plant are used as an antidiabetic agent in Nigerian folk medicine, although when aqueous extract given orally did not alter blood glucose level in either normoglycemic or streptozotocin induced diabetes. The aqueous extract of *Mangifera indica*

possess hypoglycemic activity. This may be due to an intestinal reduction of the absorption of glucose.

Holy Basil (Ocimum sanctum): It is commonly known as Tulsi. Since ancient times, this plant is known for its medicinal properties. The aqueous extract of leaves of *Ocimum sanctum* showed the significant reduction in blood sugar level in both normal and alloxan induced diabetes. Significant reduction in fasting blood glucose, uronic acid, total amino acid, total cholesterol, triglyceride and total lipid indicated the hypoglycemic and hypolipidemic effects of tulsi in diabetes

Bhuiawala (Phyllanthus amarus): It is commonly known as Bhuiamala. Traditionally it is used in diabetes therapeutics. Methanolic extract of *Phyllanthus amarus* was found to have potent antioxidant activity. This extract also reduced the blood sugar in alloxanized diabetes

ANTIDIABETIC ACTIVIY ARTOCARPUS HETEROPHYLLUS

Hot water extract of mature jack leaves (*artocarpus heterophyllus* lam, family: moracea) is recommended by ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus [69]. Previous studies have indicated that an extract of *artocarpus heterophyllus* improves the glucose tolerance in normal human subjects and diabetic patients [70] literature survey reveals that aqueous extract of *artocarpus heterophyllus lam*. Leaves shown to have anti-diabetic activity when investigated in rats[68].moreover flavonoid fraction of *Artocarpus heterophyllus* leaf shown to have hypoglycemic action [71].An ethyl acetate fraction of *A. heterophyllus* leaf extract exerts strong hypoglycemic activity in both normoglycaemic and diabetic rats[72].Jack Fruit n-butanolic extracts exert hypoglycemic and hypolipidemic effects in STZ-diabetic rats through an antioxidative pathway that might be referred to their flavonoid contents.[73]

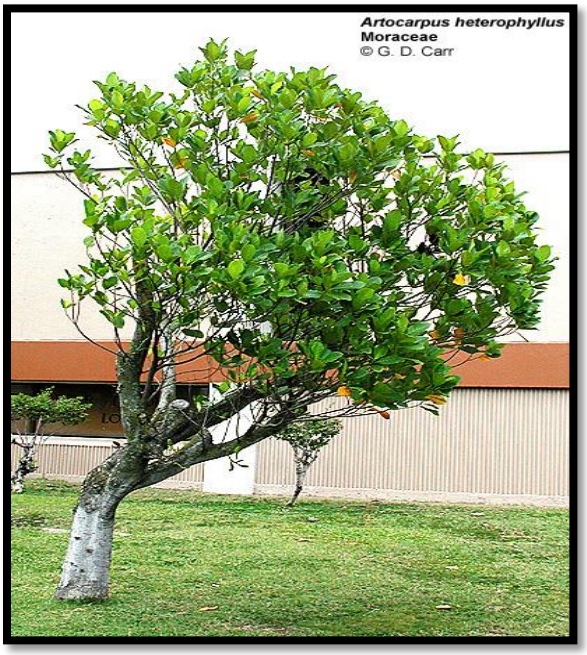
ANTIDIABETIC ACTIVIY OF PIPER NIGRUM

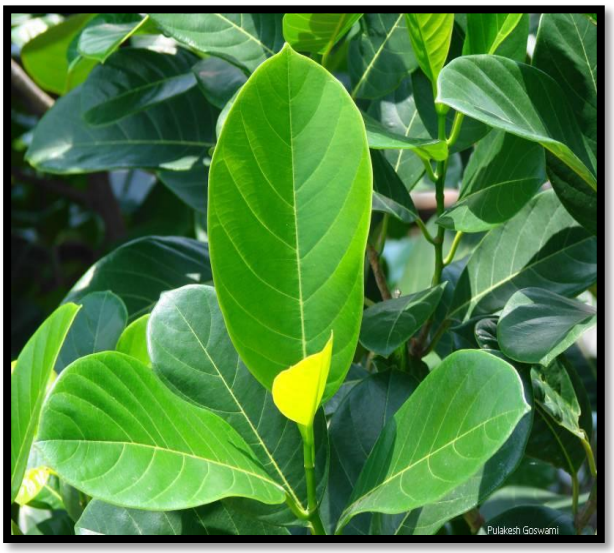
Ethanolic Leaves Extract of Black Pepper (*Piper Nigrum*) on Alloxan-Induced Diabetic Rats shown to have Hypoglycemic Potentials [74]. Piperine has the potential to be used as an antidiabetic agent. Its acute effects of raising blood glucose could also be used beneficially by using it in combination with known antidiabetics to counteract their adverse effect of hypoglycemia. The sub-acute administration of piperine has statistically significant antihyperglycemic activity while acutely it raises blood glucose at high doses. [75] Oxidative stress plays a key role in diabetes, and treatment with *P. nigrum* and *V. rosea* are useful in controlling not only the glucose and lipid levels but these components may also be helpful in strengthening the antioxidants potential [76]

NEED OF THIS STUDY

Literature survey reveals that Artocarpus hetrophyllus and Piper nigrum are extensively used in traditional medicine and various isolated constituents from these plants are also used. However, the toxicological and antidiabetic status of their combination have not been investigated yet .the present study was planned to evaluate the toxicological and antidiabetic statues of this formulation using streptozotocin induced diabetics rat with a view to provide scientific evidence.

2. PLANT PROFILE





ARTOCARPUS HETEROPHYLLUS LAM. – JACKFRUIT

TAXONOMICAL CLASSIFICATION

Table: – Taxonomical classification of Artocarpus Heterophyllus Lam [e]

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Hamamelididae
Order	Urticales
Family	Moraceae – Mulberry family
Genus	*Artocarpus* J.R. Forst. & G. Forst. – breadfruit
Species	*Artocarpus heterophyllus* Lam. – jackfruit

Description: [f]

Jackfruit is a handsome tree that can grow up to 9-21 m (70 ft) tall, with evergreen, alternate, glossy and leathery leaves to 22.5 cm (9 in) in length. The tree is monoecious, meaning that male and female flowers appear on the same tree. Tiny male flowers are borne in oblong clusters, while the female flower clusters are rounded. The largest of all trees produces fruits that can reach 20-90 cm (3 ft) in length and weighing 4.5-20 or as much as 50 kg (110 lbs). All parts of the tree contain sticky, white latex. The fruit has a unique compound structure. The exterior of the fruit is green or yellow when ripe and composed of numerous hard, cone-like points attached to a thick and rubbery, pale yellow or whitish wall. The interior consists of large fully developed "bulbs" (called perianths) of yellow, banana-flavored flesh, massed among narrow ribbons of thin, tough undeveloped perianths and a central, pithy core. Each bulb encloses a smooth, oval, light-brown "seed" covered with a thin white membrane. The seed is thick, white and crisp within. One single fruit can have from 100 to 500 seeds inside. There is one other unique and peculiar aspect about the Jackfruit: when fully ripe, the unopened fruit emits a strong disagreeable odor, resembling that of decayed onions, while the pulp of the opened fruit smells of pineapple and banana

Growth & Distribution [104]

Jackfruit has been cultivated since prehistoric times and has naturalized itself in many parts of the tropics, particularly in Southeast Asia, where it is today an important crop of India, Myanmar, China, Sri Lanka, Malaysia, Indonesia, Thailand and Philippines. It is also grown in parts of Africa, Brazil, Surinam, Caribbean, Florida and Australia. It has been introduced to many Pacific islands since post European contact and is of particular importance in Fiji, where there is a large population of Indian descent

Phytochemistry

The *Artocarpus heterophyllus* contains various chemical constituents as several flavones colouring matters, morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone and artocarpanone [105] . The heart wood on analysis yields moisture 6.7%, glucosides 38.0%, lipids

0.7%, albumin 1.7% and cellulose 59.0 % [106] . The plant also contains free suger (sucrose), fatty acids, ellagic acid and some essential Amino acids like Arginine, Cystine, Histidine, Leucine, Lysine, Metheonine, Theonine, Tryptophan etc. [107] . Bark from main trunk contains betullic acid and two new flavone pigments, cycloheterophyllin ($C_{30}H_{30}O_7$) [108] . Triterpenic compounds like cycloartenyl acetate, cycloartenone are also reported [109] . Heterophylol a phenolic compound with novel skeleton was obtained from Artoca*ip*us *heterophyllus* [110] . There is only 3.3% tannin in the bark, which is occasionally made into cordage or cloth. The leaves and stem show the presence of sapogenins, cycloartenone, cycloartenol, β-sitosterol [111] and tannins, they show estrogenic activity. A root contains β-sitosterol, ursolic acid, Betulinic acid and cycloartenone [112] .

Jacalin, the major protein from the *Artocarpus* heterophyllus seeds, is a tetrameric two-chain lectin combining a heavy chain of 133 amino acid residues with a light *β* chain of 20-21 amino acid residues. It is highly specific for the O-glycoside of the disaccharide Thomsen-Friedenreich antigen (Gal*β*1- 3GalNAc), even in its sialylated form. This property has made jacalin suitable for studying various O-linked glycoproteins, particularly human IgA1 [113] . Jacalin's uniqueness in being strongly mitogenic for human $CD4^+$ T lymphocytes has made it a useful tool for the evaluation of the immune status of patients infected with human immunodeficiency virus HIV-1 [114] . Two novel 2', 4', 6'--trioxygenated flavanones, heteroflavanones A and B were isolated from the root bark of *Artocarpus heterophyllus* [115, 116]. Three phenolic compounds were characterized as artocarpesin, norartocarpetin and oxyresveratrol by spectroscopic methods and through comparison with data reported in the literatures [117] . The composition of carotenoids of *A.heterophyllus* is carotenes β-carotene, α - carotene, β-zeacarotene, α-zeacarotene and β-carotene-5, 6-epoxide and a dicarboxylic carotenoid, crocetin were identified [118] .

Traditional uses

The leaves are useful in fever, boils, wounds and skin diseases. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and constipating. The wood is nervine, antidiabetic, sedative and is useful in convulsions [119] . The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent [120] . The ash of Jackfruit leaves is used in case of ulcers. The dried latex yields artostenone, convertible to artosterone, and a compound with marked

androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings [121] . The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property and its pith is said to be abortifacient. Latex is used as an antiinflammatory agent [122]

PLANT PROFILE

PIPER NIGRUM L – BLACK PEPPER

TAXONOMICAL CLASSIFICATION

Table: – Taxonomical Classification of *Piper Nigrum L* [g]

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Magnoliidae
Order	Piperales
Family	Piperaceae – Pepper family
Genus	*Piper L.* – Pepper
Species	*Piper nigrum L.* – Black Pepper

GEOGRAPHY AND DISTRIBUTION [i]

Black pepper is native to the Western Ghats of Kerala State in India, where it grows wild in the mountains. It is cultivated all over the tropics as a commercial crop. Vietnam, Indonesia, Brazil and India are the major producers.

DESCRIPTION

Overview: A climber that grows to a height or length of 10 m or more. Once the main stem is established it grows many side shoots to create a bushy column. The plants form short roots, called adventitious roots, which connect to surrounding supports.

Leaves: Almond-shaped, tapering towards the tip, dark green and shiny above, paler green below, arranged alternately on the stems.

Flowers: Borne in clusters along flowering stalks known as spikes. 50–150 whitish to yellow-green flowers are produced on a spike.

Fruits: Round, berry-like, up to 6 mm in diameter, green at first but turning red as they ripen, each containing a single seed. 50–60 fruits are borne on each spike.

CHEMICAL COMPOSITION OF BLACK PEPPER [123]

Compounds responsible for odor, aroma and pungency in black pepper is found out from GC-MS analysis. It contains α-terpineol (Floral), Acetophenone (Irritant, sharp), Hexonal (Green apple), Nerol (Fresh, Floral, Herbal), Nerolidol (Mild spicy, Rooty), 1, 8–cineol (Camphory), Dihydrocarveol (Warm, Woody), Citral (Citrussy), α–pinene (Terperic, Oxidised), Piperolnol (Sweet, Floral) [124]

It contains lignans, alkaloids, flavonoids, aromatic compounds and amides [125]. It also contains essential oil up to 3.5% and this oil constitutes sabinene, pinene, phellandrene, linalool and limonene. It also has piperine which is a weak basic substance. Chavicine is an isomer of piperine [126]. Piperine and Chavicine are not responsible for the aroma of the black pepper but piperine imparts pungency to the black pepper [127, 128].

Vitamins content in black pepper: Choline, Folic acid, Niacin, Pyridoxine, Riboflavin, Thiamin, Vitamin A, and Vitamin C Vitamins A, Vitamin E, Vitamin K are the major vitamins found in the black pepper.

Minerals content of black pepper: Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Zinc are the main minerals found in the black pepper.

USES [i]

Food- This hotly pungent spice is one of the earliest known and most widely used spices in the world today. It is used as flavoring, particularly for savoury foods, meat dishes, sauces and snack foods. It is also used as a table condiment. Black pepper is also used to produce pepper oil and oleoresin, which are frequently used in the production of convenience foods and sometimes also for perfumery.

Traditional medicine- Black peppercorns feature as remedies in Ayurveda, Siddha and Unani medicine in South Asia. They are most frequently used as an appetizer and to treat problems associated with the digestive system, particularly to eradicate parasitic worms. Some traditional uses of black pepper are supported by scientific evidence.

In Ayurvedic medicine, black pepper has been used to aid digestion, improve appetite, treat coughs, colds, breathing and heart problems, colic, diabetes, anaemia and piles. Stomach ailments such as dyspepsia, flatulence, constipation and diarrhoea are all treated with black pepper.

Black pepper has been used as a remedy for cholera and syphilis. It has also been used in tooth powder for toothache, and an infusion of black pepper has been suggested as a remedy for sore throat and hoarseness. Black pepper may be chewed to reduce throat inflammation. Externally, it has been applied as a paste to boils and to treat hair loss and some skin diseases. Oil of pepper is reputed to alleviate Black pepper has been given by inhalation to comatose patients. It is also believed to be useful against hepatitis, urinary and reproductive disorders.

In Unani medicine, black pepper has been described as an aphrodisiac and as a remedy to alleviate colic.

Black pepper contain compounds called alkaloids. One of these is piperine, reported to act as a central nervous system depressant and to have anti-fever, pain-relieving, anti-inflammatory and insecticidal effects. Some experiments suggest black pepper and its constituent piperine may have potential in the treatment of vitiligo (loss of skin pigment) as it helps increase pigmentation in the skin. Black pepper is also reported to have anti-fungal and anti-oxidant properties. [i]

3. LITERATURE REVIEW

Nazli shahin *et al.*, 2012 Investigated the antidiabetic property of the aqueous leaves extract of Artocarpus. *Heterophyllus* at dose of 250 mg/kg for 21 days to diabetic rats. The study showed significant reduction of serum glucose, total cholesterol, whereas significant increased level of high density lipoprotein. The leaf extract showed antidiabetic and Antihyperlipidemic by restoration of blood glucose level and biochemical profiles. [81]

Chandrika U. G *et al.*, 2006 Reported that flavonoid fraction of hot water extract of mature jack leaves (*Artocarpus heterophyllus*) caused the hypoglycemic effect at a dose of 50 mg/Kg, both in normal and alloxan-diabetic rats. The hypoglycemic effect of the flavonoid fraction of jack leaf (49%) is higher than that of tolbutamide (27.0%). Administering the flavonoid fraction for 3 months had no significant effects on liver function while the histology of liver, kidney and heart revealed no damage. [82]

Om Prakash *et al.*, 2013 Evaluated the Analgesic and Immunomodulator activity of methanolic and aqueous leaves extract of *Artocarpus heterophyllus* Lam by using Eddy's hot plate method and Swimming endurance test at the dose levels of 250 and 500mg/kg in Swiss albino mice respectively.Studies indicate that the methanolic extract of the leaves of *Artocapus heterophyllus* possess analgesic and immunomodulator activity up to significant level which is justified by Eddy's hot plate and Swimming endurance test.[83]

Efrilia Tanjung *et al.*, 2015 Perfomed the antidiabetic and antioxidant activity of aqueous extract of Jackfruit.The antidiabetic activity were determined by inhibition of haemoglobin glycation method. Phytochemical constituent like ascorbic acid, β-carotene and lycopene also determined. Antioxidant activity was measured by hydroxl radical and hydrogen peroxide scavenging activity, and chellating effect of ferrous iron [84]

Sureka Chackrewarthy *et al.*, 2012 Investigated Hypoglycaemic and Hypolipidaemic Effects of an Ethylacetate Fraction of Artocarpus heterophyllus Leaves. The leaf extract exerts strong

hypoglycaemic activity in STZ induced diabetic rats when compared with a standard drug Glibenclamide (0.6 mg kg^{-1} bw^{-1}) and it also show improvement in the hyperlipidaemia by lowering of serum total cholesterol and triglycerides [d]

Meshram R. L *et al.*, 2011 Investigated comparative evaluation for in vitro Antioxidant activity from Artocarpus heterophyllus Lamk and alanites aegyptiaca L.The antioxidant activity is 12.34 % for acetone extract from the seed of Artocarpus whereas, chloroform extract of the same showed 14.87% inhibition.[85]

Sindhu.S.Nair *et al.*, 2013 Evaluated the In Vitro Anti diabetic Activity methanolic extracts of bark of Cinnamomum zeylanicum, , leaves of Piper betle, leaves of Artocarpus heterophyllus and fruit of Artocarpus altilis.The results of the work indicate that the selected plants possessed considerable invitro anti diabetic activity[86]

Anjali Ruikar *et al.*, 2015 Investigated the antioxidant potential of ethanolic extract of bark of Artocarpus heterophyllus. DPPH radical scavenging assay and Superoxide radical scavenging assay were performed using ascorbic acid as standard. Hydroxy radical scavenging assay, Nitric radical assay and Hydrogen peroxide assay were studied with curcumin as standard.Reducing power assay was carried out with BHT as standard.Trolox Equivalent Antioxidant capacity was also investigated. The results of different in vitro antioxidant activity assays indicated that the bark extract possessed appreciable free radical scavenging effects. [87]

Haidy S. Omar *et al.*, 2011 Investigated the potential hypoglycemic and hypolipidemic activities of *Artocarpus heterophyllus* (jack fruit) leaf extracts of both 70% ethanol extract and n-butanol extracts and compared to the hypoglycemic reference drug Glibenclamide (600 µg kg^{-1} day^{-1}). Diabetes was induced in the rats by a single intraperitoneal (i.p.) injection of freshly prepared STZ (60 mg/kg b. w.) in normal saline He conclude from this study that ethanolic and butanolic extract exert hypoglycemic and hypolipidemic effects in STZ-diabetic rats through an antioxidative pathway that might be referred to their flavonoid contents[88]

M. Kaleem *et al.*, 2005 Orally administered the aqueous extract of Piper nigrum seeds and Vinca rosea flowers to alloxan induced diabetic rats once a day for 4 weeks. These treatments lead to

significant lowering of blood sugar level and reduction in serum lipids suggesting that treatment with this drug are useful in controlling not only the glucose and lipid levels but these components may also be helpful in strengthening the antioxidants potential[89]

Onyesife *et al.*, 2014 Investigated hypoglycemic activity of ethanolic extract of piper nigrum leaves on alloxan induced diabetics rats for a period of 21days and the result suggest that the piper nigrum leaves possess anti-diabetic properties[90]

Shanmugapriya K *et al.*, 2014 Studied the activity of ethanolic extract of *Piper nigrum* leaves against Dalton lymphoma ascites in Swiss albino mice. Phytochemicals analysis, Antimicrobial and antioxidant activities were evaluated by the standard methods. Anticancer activity was evaluated by liver histopathology, serum biochemical parameters.The present study suggested that anti-cancer activity of ethanolic extract might be mediated through scavenging of free radicals and it has a significant potential to use as a natural antioxidant agent due to its significantly higher amount of phenolic content. [91]

Gayatri Nahak *et al.*, 2011 Evaluated Phytochemical and a comparative study of the Antioxidant activity of ethanolic and methanolic extracts of dried fruits of *Piper cubeba* and *Piper nigrum.* The results of antioxidant activity indicate that higher free radical scavenging activity in ethanolic extracts of *Piper cubeba* in comparison to *Piper nigrum* due to presence of phytochemical constituents especially polyphenols.[92]

K Shanmugapriya *et al.*, 2012 Evaluated antioxidant activity and antimicrobial activity against some pathogenic microbes. The result revealed that the leaf extract have potential antioxidant activity which is determined by free radicle scavenging method which is due to the presence of flavonoid. The extract also have activity against gram positive and gram negative bacteria and also against fungi which is evaluated by disc diffusion method. [93]

O. Onyesife Chioma *et al.*, 2014 Evaluated the lethal toxicity and histology assessment of the organs of diabetic rats treated with different doses ethanolic extract of *Piper nigrum* was analyzed. The acute toxicity (LD_{50}) test result showed that the leaves were not toxic up to 5000mg/kg body weight after 24 h of constant observation. The histological sections of the liver and kidney of extract treated rats showed no remarkable histological changes compared to Diabetic untreated

which is an indication that *Piper nigrum* extract is safe and possess no threat to the organs of metabolism. [94]

Divya Pingili *et al.*, 2012 Investigated, methanolic and hydro alcoholic polyherbal extracts having bark of Terminalia *arjuna*, *Piper nigrum* Linn seeds And *Cuminum cyminum* Linn seeds for their *in vitro* antioxidant activity by DPPH and superoxide radical scavenging method and *in vitro* antidiabetic activity by α glucosidase and α amylase inhibitory method; and anticancer activity by MTT assay against A549 human lung cell carcinoma. Among the evaluated six polyherbal extracts for anticancer activity, the combination of methanolic extract of *Terminalia arjuna* and piperine (S1+ P2) treatment have showed potent antitumor property than the standard Cisplatin and it may be due to the enhanced bioavailability of the species when given in combination[95]

Shubham Atal *et al.*, 2012 Studied the the effect of Piperine *per Se* on blood glucose level in Alloxan-Induced Diabetic Mice.The result revealed that piperine has potential antidiabetic activity, if given repeatedly at the appropriate doses over a period of time. Its acute effects of raising blood glucose could also be used beneficially by using it in combination with known antidiabetics to counteract their adverse effect of hypoglycemia. [96]

O. Onyesife *et al.*, 2014 Evaluated the Serum Lipid Peroxidation and Antioxidant Enzymes Activities in Diabetic Rats using ethanolic extract of piper nigrum leaves. The results confirmed that the untreated diabetic rats were subjected to oxidative stress as resulting in low superoxide dismutase, catalase and glutathione activities. The ethanol extract of *Piper nigrum* leaves shown to increased activities of superoxide dismutase and catalase and glutathione levels of the diabetic rats after 21 days treatment. [97]

Popi patilaya *et al.*, 2012 Studied Effects of standardized fractions of *piper nigrum* on the growth of *mycobacterium tuberculosis* H37rv strain using tetrazolium micro plate assay method to determine the minimum inhibitory concentration. The results reveals that the ethyl acetate fraction of *piper nigrum* leaf has potential activity against *mycobacterium tuberculosis*. The ant mycobacterial activity of the fraction might be through the disruption of the cell wall of mycobacteria [98]

A.Dinakar, P.D *et al.*, 2010 Studied the inhibition of thioacetamide –induced liver Fibrosis using *piper nigrum Linn seeds.* The extract reduced the level hydroxyproline which is a good marker of fibrosis and the extract show anti fibrotic activity [99]

S.K. Shiva Rani *et al.*, 2013 Evaluated the antimicrobial activity of piperine isolated from piper nigrum seeds. In the present study the antibacterial activity was measured by agar well diffusion method and antifungal activity by poisoned food technique. Piperine showed antimicrobial activity against all tested bacteria and fungus suggesting the use of piperine as antimicrobial agent [100]

Kaleem M *et al.*, 2008 Investigated the *Annona squamosa* extract on certain biochemical markers in streptozotocin (STZ) induced diabetes mellitus in rats by a single intraperitoenal (i.p.) injection of freshly prepared STZ (55 mg/kg body weight of rats) in 0.1 M citrate buffer (pH 4.5). The results shown that extract has an antihyper glycaemic effect and consequently may alleviate liver and renal damage associated with STZ-induced diabetes mellitus in rats [101]

Shaik Abdul Nabi *et al.*, 2013 Evaluated Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats by intraperitoneal administration of STZ (single dose of 50 mg/kg b.w.) dissolved in freshly prepared 0.01M citrate buffer, pH 4.5. From the results it is concluded that the plant extract is capable of managing hyperglycemia and complications of diabetes in STZ induced diabetic rats [102]

C. Hari kumar *et al.*, 2010 Evaluated the antidiabetic activity using the extracts seeds of *Eugenia jambolana*, fruits of *momordica charantia*, and leaves of *ocimum sanctum* in the ratio 1:1:1 to get a polyherbal Preparation. Treatment with the polyherbal Preparation for 11 days in diabetic animals has shown significant decrease in serum glucose, AST, ALT and biochemical parameters (urea, creatinine, triglycerides and cholesterol) levels in comparison to control animals [103]

4. AIM AND OBJECTIVE

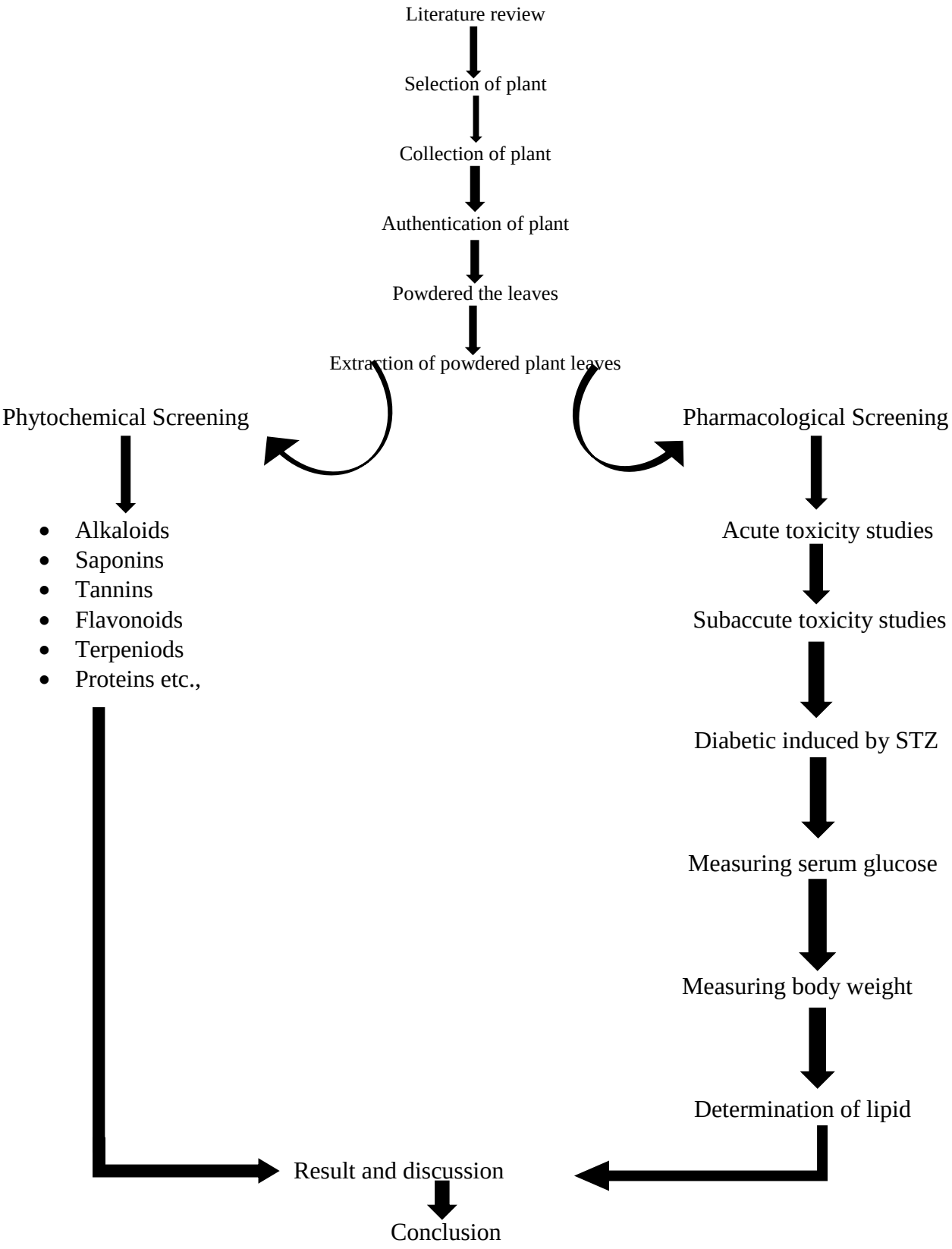
AIM

To assess the antidiabetic and toxicological studies of the combination of Piper nigrum and Artocarpus hetrophyllus. To achieve this primary aim we set a following objective.

OBJECTIVE

1. To evaluate the taxonomical studies
2. To study the pharmacognostical property of Piper nigrum and Artocarpus hetrophyllus
3. To evaluate the various toxicological study –accute and sub acute toxicity-by animal models
4. To evaluate the antidiabetic activity of the combnation of Piper nigrum and Artocarpus hetrophyllus by stz induced animal models

5. PLAN OF WORK



It is divided into following phase-

PHASE I: Taxonomical studies.

- ✓ Collection of plants.
- ✓ Authentication of plants.
- ✓ Powdered the leaves materials

PHASE II: Pharmacognostical studies.

- ✓ Successively solvent Extraction.
  - Alcohol (ethanol).
- ✓ Preliminary Phytochemistry screenings.
  - Alkaloids
  - Saponins
  - Tannins
  - Amino acid
  - Flavonoids
  - Terpenoids
  - Protein
  - Steroids

PHASE III: Pharmacological studies.

- ✓ Acute toxicity studies (as per OECD guidelines)
- ✓ Sub acute toxicity studies (as per OECD guidelines)
- ✓ Induces diabetic in rats by STZ

- ✓ Measuring the body weight
- ✓ Measuring the water intake
- ✓ Measuring the food intake
- ✓ Determination of lipids
    - Triglyceride
    - Total cholesterol
    - HDL
    - VLDL

PHASE IV: Result and Documentation.

Evolution of statistical significance result by computer aided program and systemic documentation. Values were presented as mean SEM Data were analyzed using of variance (ANOVA) and group means were compared with Turkey's post hoc Multiple Comparison Test using SPSS software version 17. $P<0.05$ is considered as significant.

6. MATERIALS AND METHOD

Chemicals:-

- Streptozotocin
- Glibenclamide
- Petroleum ether
- Ethanol
- Benzene
- Chloroform
- Studies were carried out in albino rats.

Instruments:-

- Soxhlet Apparatus
- Hot air oven
- Actophotometer
- Rotarod Apparatus
- Analgesiometer
- Glucometer

TAXONOMICAL STUDIES.

1) **Collection of plants.**

Leaves of Artocarpus Heterophyllus and Piper Nigrum were collected from the region of kanjrapally, kottayam in Kerala, India.

The freshly collected leaves were thoroughly cleaned and soaked in fresh water repeatedly to separate mud particles sticking on to plant constituents. The plants materials collected were cut into small bits of about 2-3 in size. The leaves were powdered with a mechanical grinder and this powder was subjected to various studies for which the materials and methods which is presented below.

2) Authentication of plant.

The dried whole plant of *Artocarpus Heterophyllus* and *Piper Nigrum* were authenticated by Dr. M. V. Krishnaraj, Department of Botany, Baselius College Kotayam

PHARMACOGNOSTICAL STUDIES.

EXTRACTION PROCEDURE.

Figure : Assembly extraction Apparatus

The crude weighed powder were mixed and placed in the Soxhlet apparatus by using ethanol. From the 500gm of crude powder was extracted with 2.5 liter of ethanol (60 – 80) by continuous hot percolation using Soxhlet apparatus. This can be continued up to 24 hours. After the completion of extraction process, Soxhlet is removed and the residue obtained is stored in the dissector.

PHYTO CHEMICAL SCREENING[129-131]

The plant may be contain the compound such as carbohydrate, protein, and lipids. It also contains the compound like. Tannins, glycosides, alkaloids, Volatiles oils and other important compounds which are utilized by man as food and medicines. These compounds are responsible for its medicinal properties

TEST FOR CARBOHYDRATES

Molish test:-

The powdered sample was added with 1 ml of alpha napthol solution along with conc Sulphuric acid solution in the test tube, reddish colour was produced at the junction between 2 liquid shows the presence of carbohydrate.

Fehling test:-

To the powdered sample was added with both Fehling A and Fehling B solution and placed in the water bath for a sufficient time. This shows the brick red colour. It shows the presence of carbohydrate.

Benedicts test:-

To the powdered sample add 8 drops of benedict's reagents and boil the sample vigorously for 5 min, it shows the red ppt. indicating the presence of carbohydrate.

TEST FOR ALKALOIDS

Powdered sample was taken in a test tube and add few drops of hydrochloric acid and filtered. The filtrate was tested with various alkaloid agents,

Mayer's reagents:-

To the above filtrate add small quantity of Mayer's reagent to form cream precipitate. This shows the presence of alkaloids.

Dragendorffs reagents:-

To the above filtrate add small amount of Dragendorffs reagent and it forms an orange brown precipitate. This shows the presents of alkaloids.

TEST FOR FLAVONOIDS

The plant extract is filtered and add 5 ml of dilute ammonia solution and followed by the addition of concentrated sulphuric acid. It forms a yellow colour. It shows the presence of flavonoids.

TEST FOR STEROIDS

Salkowaski test:-

A small volume plant extract is mixed with chloroform and the same volume of sulphuric acid is added on it. Cherry red colour was obtain in the chloroform layer. This shows the sample contain steroids.

Libbbermann burchatd test:-

The extract is dissolved in 2 ml of chloroform, 10 drops of acetic acid and conc. Sulphuric acid were added. The solution becomes reddish colour then it turns to bluish green colour. This shows that the plant extract has the presence of steroids.

TEST FOR TANNINS

Few amount of plant extract is treated with vanillin hydrochloric acid reagent. It forms, pink or red colour due to the formation of phloroglucinol, it indicate the presence of tannins.

TEST FOR PROTEIN

Mellon's reagents:-

Mellon's reagents (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

Ninhydrin Test:-

Add 2 drops a freshly prepared 0.2% ninhydrine reagent to the extract and heated. Development of blue colour may indicate the presence of peptide, amino acid (Protein).

TEST FOR GLYCOSIDES

Keller- killani test:-

To the small quantity of extract, acetic acid was dissolved and adds few drops of ferric chloride and transferred to the surface of conc Sulphuric acid. At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

TEST FOR SAPONINS.

Foam test:-

1 ml of extract solution is diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of Saponins.

TOXICOLOGICAL STUDIES.

TOXICITY STUDY [132].

ACUTE TOXICITY STUDY

Experimental Protocol

Guideline	: OECD-423
CPCSEA Ref. No	: IAE1012/c/06/CPCSEA
Test	: Limit test
Species	: *Rattus norvegicus*
Strain	: Albino Wistar rats
Number of animals	: 10 animals (5 for each extract)
Sex	: Female
Initial dose	: 200mg/kg
Route of administration	: Oral
Duration	: 4 hr close observation, followed by 14 days observation
Others	: Body weight, mortality status
Parameters	: CNS, ANS and behavioral changes
Blood collection	: Not needed
Sacrifice	: On day 14 after oral administration

Table 6:- EXPERIMENTAL DESIGN FOR ACUTE TOXICITY STUDY

GROUP	**DOSE (mg/kg)**
GROUP 1	*50*
GROUP 2	*200*
GROUP 3	*2000*
GROUP 4	*Control*

STUDY DESIGN

Selection of Test animal

Female adult Wistar rats of 8-12 weeks are selected. Nulliparous and non-pregnant animals were obtained from the centralized animal house of RVS College of Pharmaceutical sciences, Sulur and they are acclimatized for holding one week prior to dosing.

Housing and feeding conditions

Temperature - As per OECD GUIDELINE-420 the temperature of animal house is maintained at $22^0C \pm 3^0C$.

Humidity - The relative humidity of animal room maintained at 50-60% preferably not exceeds 70% (OECD guidelines-420, 2001). Otherwise there may be chances of developing lesions such as ring tail and food consumption may be increased.

Light – The sequence of light used is 12 hrs light and 12 hrs dark.

Caging – Polypropylene cages with solid bottom and walls. The lids are made up of stainless steel grill which is capable to hold both feed and water.

Feeding condition and feed – Sterile laboratory feed (*ad libitum*) and water daily. The feed used is brown coloured chow diet.

Drug Administration

Animals are fasted prior to dosing (food but not water should be withheld for overnight).After that animals are weighed and the test substance administered. The healthy rats has been taken and divided into 4 different groups. The ethanolic extract of the leaves of *Artocarpus Hetrophyllus* and leaves of *Piper Nigrum* were mixed in the ratio 1:1. Then the extract was dissolved in ethanol. The test substance is administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

Clinical observation

All animals were monitored continuously with special attention for 4 hrs after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioral or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed and then humanely killed and LD50 values are established.

SUB ACUTE TOXICITY STUDY

Experimental Protocol

Guideline : OECD-407

CPCSEA Ref. No : 1012/c/06/CPCSEA

Species : *Rattus norvegicus*

Strain : Albino Wistar rats

Number of animals : 10 for each groups

Sex : Male/Female

Route of administration : Oral

Duration : 28 days

No. of blood collection : 2

Duration of blood collection : 0th day, 28th day

Blood collection route : Retro orbital

Sacrifice : After 28 days of oral administration

Body weight recording : weekly intervals

Table 7:-EXPERIMENTAL DESIGN FOR SUBACUTE TOXICITY STUDIES

GROUPS	DOSE	NO. OF ANIMALS
GROUP 1	High dose	10(5M+5F)
GROUP 2	Low dose	10(5M+5F)
GROUP 3	Medium dose	10(5M+5F)
GROUP 4	Control	10(5M+5F)

STUDY DESIGN

Selection of Animals

Male and Female rats were selected and are acclimatized for 5 days prior to the start of study. The females are nulliparous and non-pregnant. At the commencement of study the weight variation of animals used minimal and not exceed ± 20 % of the mean weight of each sex. Repeated dose oral study was conducted as a preliminary to a long term study preferably animals from the same strain and source were used in both studies.

Housing and feeding conditions

The temperature in the experimental animal room was maintained at 22^0C $\pm 3^0$C. The relative humidity was 50-60% (not exceed 70%) and the lighting sequence was 12 hrs light and 12 hrs dark. For feeding, conventional laboratory diet was used with an unlimited supply of drinking water. Animals were housed in small groups of same sex (NMT 5 animals in each cage)

Preparation of animals

Healthy young adult animals were randomly assigned to control and treatment groups. Cages were arranged in such a way that possible effects due to cage placement were minimized. The animals were identified uniquely and kept in their cages for five days prior to the start of the study to allow for acclimatization to the laboratory conditions.

TABLE 8:-DOSE OF DRUG FOR SUB ACUTE TOXICITY STUDIES

GROUPS	DOSE (mg/kg)
GROUP 1	*100*
GROUP 2	*200*
GROUP 3	*400*
GROUP 4	*Control*

Dose Administration

The leaf extracts were administered by oral gavages.The ethanolic extract of the leaves of *Artocarpus Hetrophyllus* and leaves of *Piper Nigrum* were mixed in the ratio 1:1. Then the extract was dissolved in ethanol. Ten animals (5 males and 5 females) were used at each dose level for

each extracts. Three test groups and a control group were used for both extracts and the highest dose level was chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter a descending sequence of dose levels selected with a view to demonstrating dosage related response and non-observed-adverse effects at the lowest dose level (NOAEL). The animals were dosed with test substance daily 7 days each week for a period of 28 days.

FUNCTIONAL OBSERVATIONAL BATTERY (FOB) OBSERVATIONAL PROCEDURES

Home-cage observation

The animals were observed closely from outside the cages without agitating them; body position, respiration, clonic involuntary movement, tonic involuntary movement, vocalizations and palpebral closures were noted [133].

Hand-held observations

The reactions of the animals were observed when they were removed from their cages and held. Reactivity, palpebral closure, lacrimation, salivation and pilo erection were noted. Detail any finding related to dirtiness of hair cast, bite marks, missing nails, gauntness (stomach could be touched, median vertebrae protrude) or death (findings) [134].

Open field activity

Each animal was gently placed in the centre of a field and made the following 10 observations for 3 minutes [135]

1. Rearings
2. clonic involuntary movement
3. Tonic involuntary movement
4. Gait
5. Movements
6. Arousal
7. Stereotype
8. Abnormal behavior
9. Defecations
10. Urinations.

Stimulus response

Approach response; touch response, eyelid reflex, pinna reflex, sound response, tail pinch response, tail flick latency, pupillary reflex, righting reflex observations were carried out:

Eyelid reflex assessment

The animal's eyelid was touched with a dull object (cotton buds) and blinking response was noted.

Pinna reflex assessment

This is to assess the sensor motor activity of the animals. The fine object (cotton) was slightly touched inside the ear of the animals. The time duration taken for the response was noted.

Sound response assessment

In order to assess the sensor motor activity of the animals, the fingers snapped above and behind the animals head. The time duration taken for the flinch and flicks were scored.

Pupillary response assessment

The pen light was shined in the eyes of the animals. The response to contraction was scored.

Response to visual stimuli

The blunt object was approached the animals head and held the object 1 to 2 inch from the animal's head for few seconds. The time duration taken for approaching the object was recorded.

Tail flick latency assessment

This method was used to assess the sensor motor status of the animals [133] Response to painful stimuli was measured by the tail flick method using analgesiometer. In this the animals were exposed to noxious stimulus like radiant heat and tail flick latencies (the time required for the flicking of tail i.e. the reaction time and a mean of two pre drug recordings were taken as basal value(0minute). A cut off time of 10 seconds was maintained in order to prevent tissue injury (based on the reaction time that generally varied between 3-4 seconds).

Tail pinch response

This was used to assess the sensor motor activity of the animals and the response to painful stimuli was measured in this method. The animal's tails were lightly pinched approximately 2 inch from the tip with the help of forceps. Recordings were noted.

NERVOUS AND MUSCLE MEASUREMENTS

Motor coordination assessment

For the evaluation of coordination and balance, the rotarod test was carried out. The apparatus consists of a horizontal metal rod positioned 25cm above a switch floor. The rod was divided in to

four parts by plastic plates so that four rats could be tested at a time. Rats were positioned on the rod rotating at a constant speed of 20 rpm and the time the rat could stay on the rod without falling was recorded [136].

Locomotor activity assessment

Locomotor activity is determined by using actophotometer and this is to assess the neuromuscular status of the animals. The animals were placed on the digital actophotometer. The locomotion of the animals was recorded.

Righting reflex assessment

This is also used to assess the neuromuscular status of the animals. In this the animals are placed on their back. The ability to regain a position on all four legs was recorded.

Landing foot splay assessment

This method was used to assess the neuromuscular status of the animals. The animals were grasped by the scruff of the neck and base of the tail. The paws were marked with ink and held above the bottom at a height of 1 ft. The animals were released and the distance between the heels was measured. The foot play distance was measured.

BODY WEIGHT

The body weights were recorded at the study Day 0, weekly during the study and at scheduled necropsy.

FEED AND WATER CONSUMPTION

Food consumption was assessed on a daily basis by weighing the feeders and expressed as grams per rat per day. Water consumption was measured.

HEMATOLOGY AND BIOCHEMISTRY

A complete battery of Haematological, clinical chemistry, and urinalysis measurements were taken at baseline and during the final week of the study on the animals per sex from each dose group. The selected animals were placed in metabolic cages, during which time urine was collected for urine analysis. Blood was also collected from the animals for fasting glucose analysis.

Haematological parameters evaluated included haemoglobin, packed cell volume (PCV); erythrocyte count, leukocyte count (total and differential), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated and reported. Clinical chemistry parameters evaluated on separated

serum samples included alkaline phosphates (ALP), SGOT, SGPT, Albumin, Protein, glucose, urea, creatinine, and serum TSH level.

All surviving test and control animals were sacrificed at study termination by cervical dislocation under anaesthesia and subjected to a complete necropsy. The organs were harvested and weighed and relative organ weights (g/kg body weight) were calculated using terminal body weights.

GROSS PATHOLOGY

All animals in the study were subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes, epididymis, prostate with seminal vesicle with coagulating glands as a whole, thymus, spleen, brain and heart of all animals (apart from those found moribund and/or intercurrently killed) trimmed of any adherent tissue, as appropriate, and their wet weight taken.

The following tissues were preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain, liver, kidneys, adrenals, spleen, heart, thymus, trachea and lungs (preserved by inflation with fixative and then immersion), epididymis, prostate + seminal vesicle, lymph and peripheral nerve.

Statistical methods

Body weight data were analyzed using a one-way analysis of variance followed by Dennett's multiple comparison tests $P<0.05$ was considered to be statistically significant. One-way analysis of variance (ANOVA) was used in analyzing body weight change, organ weight, and food and water consumption.

PHARMACOLOGICAL SCREENING

Male Swiss albino rats weighing 150-200g were used for the present work. The animals used for the experiment were maintained under standard laboratory conditions in an animal house of RVS College of Pharmaceutical sciences approved by the committee for the purpose of control and supervision on experiments on animals (Ref.No: IAE1012/C/06/CPCSEA) under 12 h dark/light cycle and controlled temperature 24±20C. They had free access to food and water *ad libitum.* The animals were acclimatized to the laboratory for a period of 7 days, before the commencement of experiment.

ORAL GLUCOSE TOLERANCE TEST [136, 137]

The ability of an individual rat to handle a standard oral glucose load was evaluated by assessing the blood plasma for glucose level. Study on Oral Glucose Tolerance Test (OGTT) initially, hypoglycemic activity of herbal formulation was carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Normal control group received only vehicle (1 ml of water) and test group received the formulation in three different dose level ie low, therapeutic and high dose respectively. Following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Dr.Morepen Gluco One, Morepen Laboratories Limited, Delhi, India).

ANTIDIABETIC ACTIVITY

INDUCTION OF DIABETICS [138-140,i]

Six adult albino rats weighting 170-240 grams were used for inducing diabetes. The animals were injected by Streptozotocin at the dose of **60 mg/kg** of the body weight by intraperitoneally administration dissolved in freshly prepared 0.01M citrate buffer, pH4.5. Streptozotocin induces diabetes within 3 days by destroying the beta cells. Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Food consumption was measured in terms of (gram), water consumption was measured in terms of (ml) and urine volume was measured in terms of (ml) on a daily basis while every 2-4 weeks in 80 days the levels of C-peptide, insulin and glucose in blood serum were also measured, so that chemical diabetes was verified in rats injected with Streptozotocin

ASSESSMENT OF DIABETIC

Diabetic was conformed after 48 hr of streptozotocin injection, the blood samples were collected through retro orbital puncture and plasma glucose level were estimated by Dr.Morepen Gluco One Glucometer. The rat having fasting plasma glucose levels more than 250 mg/dL were selected and used for this study.

PROTOCOL OF EXTRACT ON STREPTOZOTOCIN INDUCED DIABETIC RATS.

The albino rats on either sex have been selected for the experimental study. The weight should be around 170-240 gm. The animals are divided into four groups. Each group has 6 animals. Group 1 was kept as normal (normal rat) received only distilled water; group 2 was kept as negative control, Streptozotocin induced and received only water; Group 3 was treated with glibenclamide (60mg/kg) and Group 4 is diabetic induced were treated with 200mg/kg ethanolic leaves extract of both *Artocarpus Hetrophyllus* and *Piper Nigrum* in the ratio 1:1 dissolved in ethanol (100 mg ethanolic leaves extract of *Artocarpus Hetrophyllus*+ 100 mg ethanolic leaves extract of *Piper Nigrum* dissolved in ethanol)

Table 9:-Protocol of Antidibetic Study of Extract

GROUPS	***TREATMENT***
GROUP 1	***Normal Control***
GROUP 2	***Diabetic Control***
GROUP 3	***Diabetic+ Glibenclamide***
GROUP 4	***Diabetic+ Extract(200 mg/kg)***

Ethanolic leaves extract (test drug) was administered for 21 days at a dose of 200mg/kg. Dried extract dissolved in ethanol given orally. The blood was collected by sinus orbital under the light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight glucose was analyzed every week and lipid and lipoprotein profile from serum (TC, TG, HDL, LDL, VLDL.) were analyzed after 21 days. This protocol performed as per CPSEA Ref No 1012/06/CPSEA

OBSERVATION

Serum glucose level estimation (initial and final)

Body weight of the albino rats (weekly once)

Water consumption (weekly once)

Lipid and lipoprotein profile

- TC
- TG
- HDL
- LDL
- VLDL

7. RESULTS AND DISCUSSION

Table 10:- Soxhlet Extraction of the Plants

Plant	Part Used	Method Of Extraction	Solvents	Weight Of Powder Taken(A)	Weight Of Product (B)	Percentage Yield (W/V)
Artocarpus hetrophyllus	leaf	Continuous Hot percolation by Soxhlet apparatus	Ethanol (60-80°C)	**500 gm**	**49.4gm**	**9. 88**
Piper nigrum	leaf	Continuous Hot percolation by Soxhlet apparatus	Ethanol (60-80°C)	**500 gm**	**78.5gm**	**15.7**

A = Weight of powder plant material

B = Weight of extract

Percentage yield = **(B/A) X 100**

DISCUSSION

The Percentage yeld of Artocarpus hetrophyllus = 9. 88

The Percentage yeld of Piper nigrum = 15.7

PRELIMINARY PHYTO CHEMICAL SCREENING.

Ethanolic leaf extract of Artocarpus hetrophyllus and Piper nigrum was subjected to various chemical test as per the standard methods mention in the chapter no 6 for the identification of the various constituents. The result of this phytochemical analysis is listed below.

Table 11:-Qualitative Phytochemical Screening of Plant Extract

Plant constituent	Ethanolic Extract of Artocarpus hetrophyllus	Ethanolic Extract of Piper nigrum
Steroids	-	+
Carbohydrate	+	-
Flavonoids	+	+
Proteins and amino acids	+	-
Glycosides	+	-
Alkaloids	-	+
Saponins	+	+
Volatile oil	-	-
Tannins	+	+
Terpenoids	-	+

"+" - Presence

"-" - Absence.

DISCUSSION

Ethanolic extract of artocarpus hetrophyllus may contain the following phytochemicals

1. **Carbohydrate**
2. **Flavonoids**
3. **Proteins and amino acids**
4. **Glycosides**

5. **Saponins**
6. **Tannins**

Ethanolic extract of artocar Piper nigrum may contain the following phytochemicals

1. **Steroids**
2. **Flavonoids**
3. **Alkaloids**
4. **Saponins**
5. **Tannins**
6. **Terpenoids**

TOXICITY STUDIES

ACUTE TOXICITY STUDIES

Acute toxicity studies on the albino rats show no morality at a dose of 2000mg/kg, during a time period of 14 days. NOAEL were not seen in the entire study period. This acute study helps to predict that it does not contain any type of toxicity and it is full safe. So 100 mg/kg b.w ($1/20^{th}$) and 200mg/kg b.w ($1/10^{th}$) and 400mg/kg ($1/5^{th}$) were selected of that dose for the further toxicological study.

SUBACUTE TOXICITY STUDIES

In-vivo sub-acute oral toxicity study were performed to evaluate the toxicities of 28 days by continuous administration of the prepared extract , as per the procedure mentioned in the section 6. Daily observations include changes in skin, fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation), central nervous system (drowsiness, gait, tremors and convulsion), body weight and food consumption. The results were summarized in table 12 to 16. At the end of the treatment, the animals were bled from the retro orbital sinus for clinical pathology assessment which included analysis of various haematology parameters and blood biochemistry

parameters. Consequently the animals were sacrificed by cervical dislocation and necropsied to facilitate gross pathological examination of organs.

TABLE 12:- EFFECT OF EXTRACT ON STIMULUS RESPONSE

Parameters	**Observations**
Approach response	Slowly approaches, sniffs and pulls back/normal
Touch response	Slowly retreats/normal
Eyelid response	Blinks/normal
Pinna reflex	Auricle twitches/normal
Sound response	Mild reaction, hears sound/normal
Tail flick response	Flicks the tail or normal
Pupillary reflex	Contracts
Righting reflex	Lands on four limbs/normal

TABLE13:- EFFECT OF EXTRACT ON AUTONOMIC OBSERVATIONS

Sl No	Parameters	Observations
1	Reactivity	Easy/normal
2	Handling	Did not resist, very easy to handle
3	Palpebral closure	Normal (eyes are open)
4	Lacrimation	No lacrimation
5	Salivation	No salivation
6	Piloerection	None/normal
7	Hair coat	Normal
8	Bite marks	None
9	Nail status	Normal
10	Rearing activity	Normal
11	Clonic involuntary movement	None
12	Tonic involuntary movement	None
13	Gait	Normal
14	Movements	Normal
15	Arousal	Normal (keeps guard up and engages in exploratory activity)
16	Stereotype behaviour (preening, squeaking, shaking head and other repetitive behavior	None
17	Abnormal behaviour (squirming, running backwards, labored movements, squealing)	None

TABLE 14:- EFFECT OF EXTRACT ON NERVOUS AND MUSCLE MEASUREMENTS

Parameters	Observations
Abdominal tone	Normal (proper hardness)
Limb tone	Normal
Motor coordination	No abnormal changes
Landing foot splay	No significant difference between control

TABLE 15:-EFFECT OF EXTRACT ON PERCENTAGE CHANGE IN BODY WEIGHT

Sex	Dose	Days				
		0	7	14	21	28
Male	Control	129.4±3.26	139.8±2.3	148.2±2.33	158.8±1.85	169.8±3.15
	Lower Dose	167.5±2.82	180±1.69	196±1.63	199.5±2.07	204±2.70
	Middle dose	215.5±2.35	222±4.31	229.5±0.89	235±1.51	231.5±1.59
	Higher Dose	222.5±4.0	217±2.55	227±2.53	231±2.42	227±1.70
Female	Control	123.9±3.20	130±3.92	139.4±3.76	150±5.15	160.2±4.32
	Lower Dose	225±2.92	221±2.43	225±4.2	222±4.16	222±3.26
	Middle dose	203±2.92	212±2.43	212±4.2	214±4.16	216±3.26
	Higher Dose	190±2.27	203±2.40	208±2.06	211±2.18	215±1.24

FIGURE14:- EFFECT OF EXTRACT ON BODY WEIGHT

TABLE 16:- EFFECT OF EXTRACT ON RELATIVE ORGAN WEIGHT

Sex	Group	Brain	Heart	Liver	Kidney
Male	Control	1.35±0.12	5.18±.12	5.63±3.4	5.73 ±.43
	Low dose	1.37±0.43	5.20±1.3	5.73±1.22	5.82±.26
	Medium dose	1.34±0.15	5.25±1.3	5.80±3.2	5.90±3.5

	High dose	1.40±2.3	5.28±2.3	5.85±1.2	5.99±4.2
Female	**Control**	1.50±1.56	5.82±.34	5.83±.45	6.12±1.3
	Low dose	1.45±2.34	5.94±1.76	5.92±2.43	6.23±.23
	Medium dose	1.40±3.53	6.15±.54	6.12±.4	5.98±2.7
	High dose	1.42±1.43	6.27±.32	5.85±6.3	6.09±6.4

FIGURE 15:- EFFECT OF EXTRACT ON ORGAN WEIGHTS

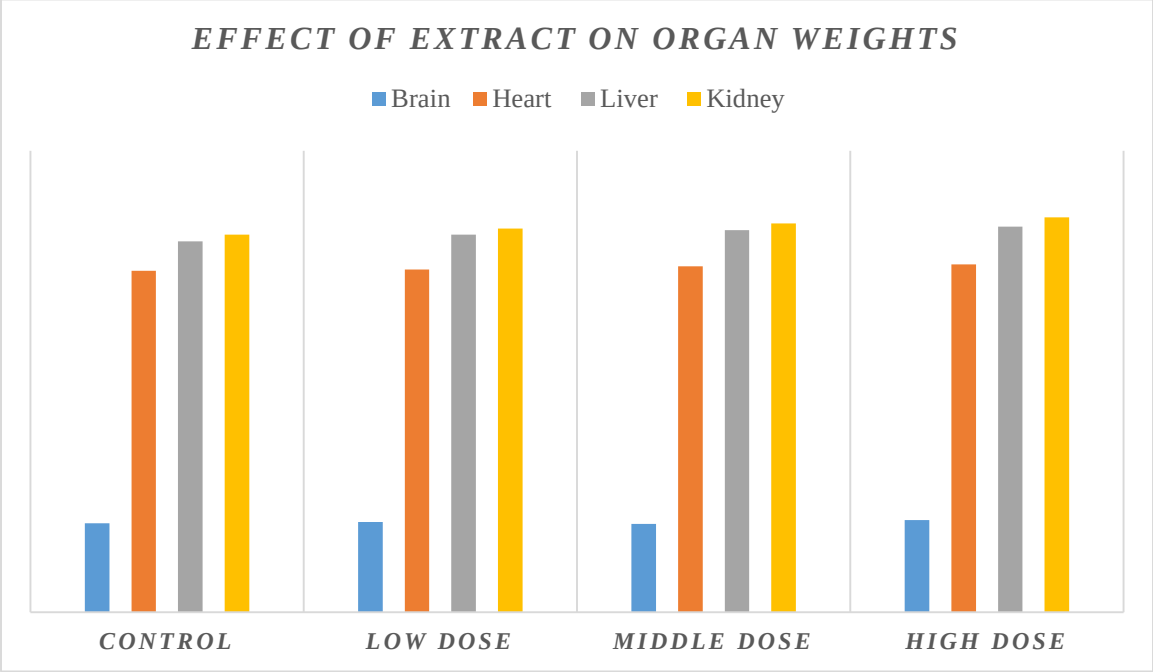


TABLE 17:-EFFECT OF EXTRACT ON SERUM BIOCHEMICAL PARAMETER

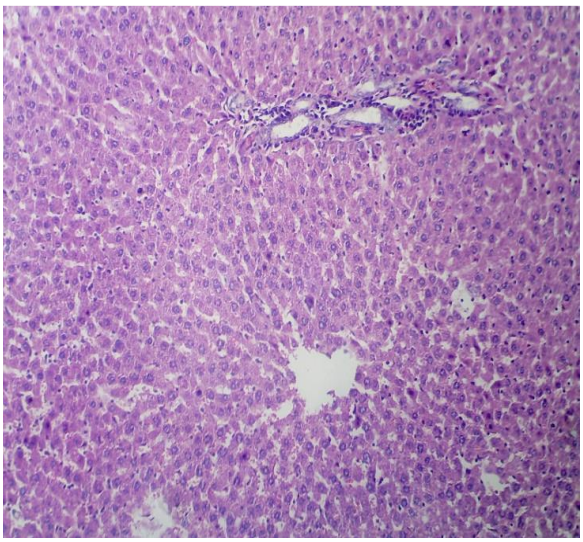
Sex	Group	Days	Hb g/dl	RBC $\times 10^6 mm^3$	WBC$\times 10^3 mm^3$
Male	Control	0	14.2±2.34	5.75±0.67	12.3±0.31
		28	13.9±1.00	5.67±0.32	11.9±.62
	Lower Dose	0	11. 8±2.3	5.9±0.65	11.83±.88
		28	9.3±2.02	4.10±0.60	13.6±.63
	Middle Dose	0	12.0±2.3	5.2±0.45	11.9±.62
		28	14.3±2.54	5.76±4.43	11±2.72
	Higher dose	0	11.9±0.57	5.3±0.15	11.6±.32
		28	14.4±2.34	5. 81±0.67	10.7±2.2
Female	Control	0	13.3±9.07	5.72±4.54	12.7±4.54
		28	14.2±1.00	5.75±0.32	12.3±1.57
	Lower Dose	0	13.9±2.3	5.73±0.65	11.9±2.2
		28	14.7±2.02	5.9±0.60	12.7±1.52
	Middle Dose	0	14.7±2.3	5.3±0.88	11.9±2.99
		28	14.5±1.25	5.7±0.45	13.1±8.6
	Higher dose	0	12.6±2.54	5.6±0.76	12.2±1.38
		28	14.5±0.57	5.9±0.15	12.9±4.2

TABLE 18:-EFFECT OF EXTRACT ON SERUM BIOCHEMICAL PARAMETER

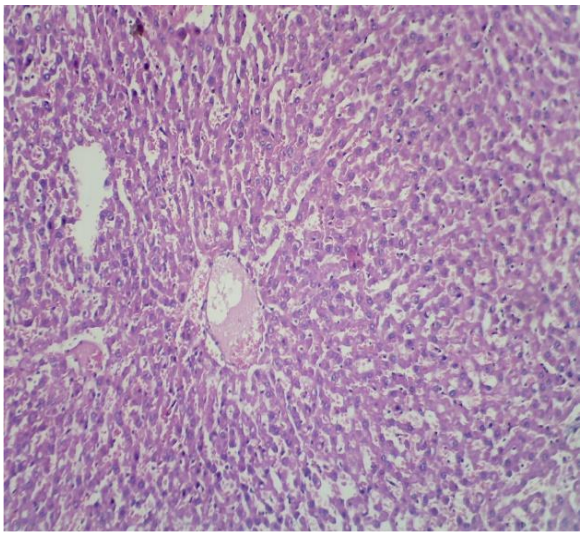
Sex	Group	Days	Serum creatinine mg/dl	Serum SGOT	Serum SGPT
MALE	Control	0	0.6±0.34	277.3±3.42	103.5±1.21
		28	0.66±0.2	277.9±5.51	103. 8±1.21
	Lower Dose	0	0.67±0.31	270.9±5.34	101. 8±5.64
		28	0.91±7.08	308.1±5.58	114.5±0.23
	Middle Dose	0	0.69±9.89	272.9±0.42	102.8±9.61
		28	0.49± 0.2	298. 8±10.39	120.9±9.61
	Higher dose	0	0.6±12.42	273.7± 0.2	103.5±15.32
		28	.68±14.26	313.6±14.17	116.5±0.28
FEMALE	Control	0	0.65±0.44	280.4±1.34	102.9±4.24
		28	0.7±0.4	282.1±5.03	102.9±1.83
	Lower Dose	0	0.63±0.20	276.9±4.13	101.6±4.7
		28	0.9±0.13	290.7±2.2	118.4±4.3
	Middle Dose	**0**	0.64± 0.42	278.6±6.4	103.6±5.56
		28	0.65± 0.2	300.7±5.4	117.9±4.32
	Higher dose	0	0.64±2.1	274.9± 0.21	103.4±3.3
		28	0.68±0.28	301.3± 0.42	114.6±5.23

HISTOPATHOLGICAL ASSESSMENT OF EXTRACT

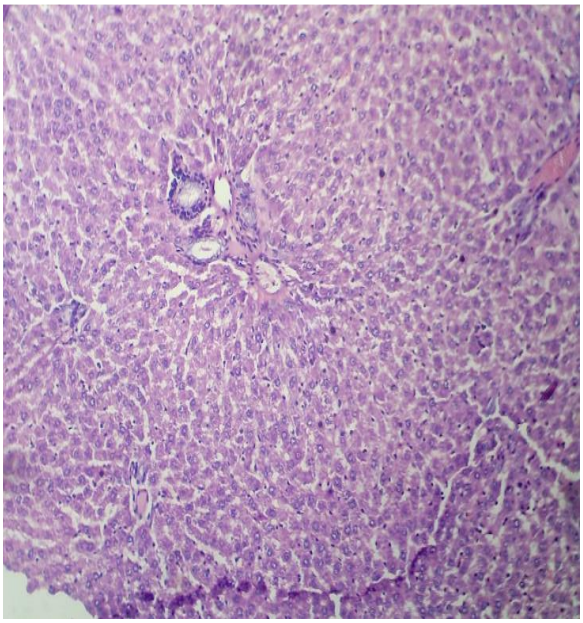
Figure 16:- T.S of Rat Liver Showing Normal Cells in Subacute Toxicity Study of Extract



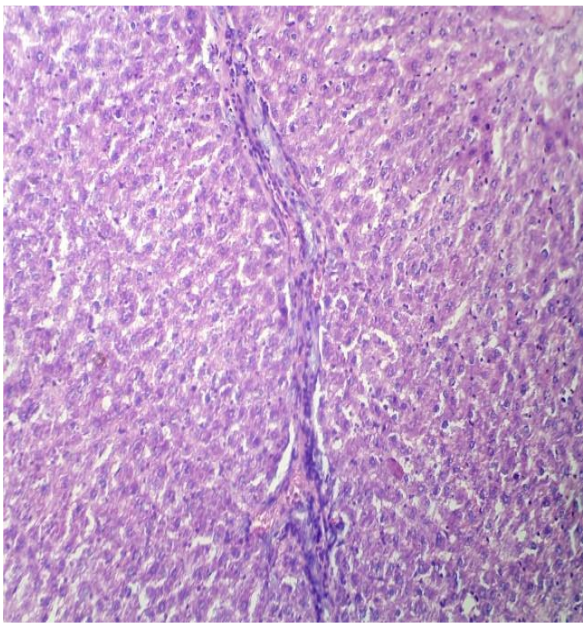
Control



Lower dose



Medium dose



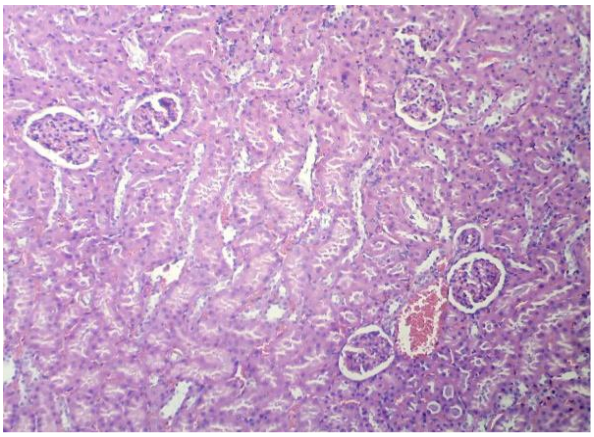
Higher dose

Microscopic appearance-

Section studies from the liver shows normal lobular architecture. Individual hepatocyte, central vein and sinusoids shows p- unremarkable. There is no evidence of inflammation and necrosis.

KIDNEY

Figure 17:- T.S of Rat Kidney Showing Normal Cells In Sub Acute Toxicity Studies of Extract



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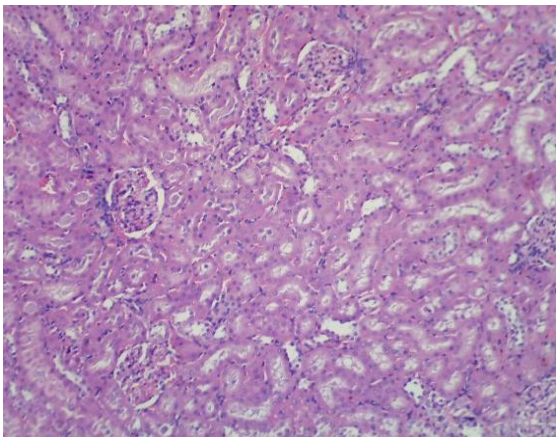
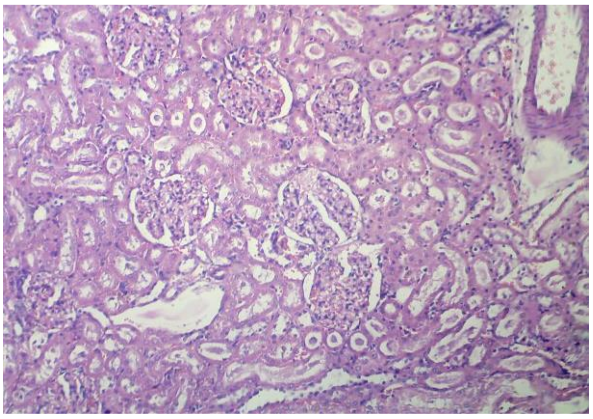
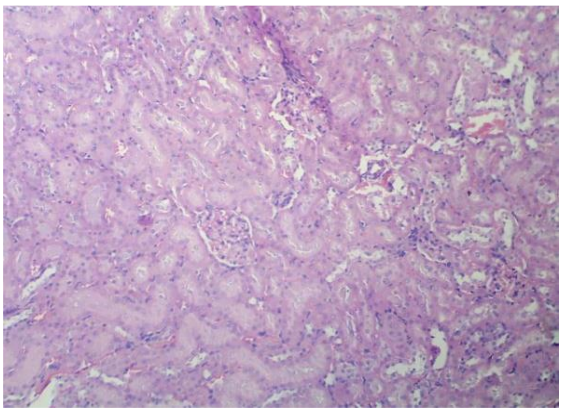


Lower dose



Medium dose



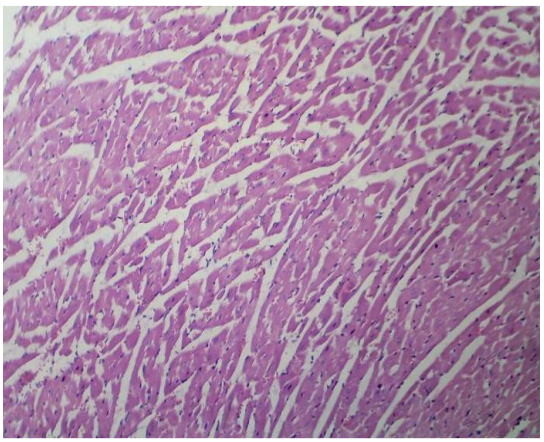
Higher dose

Microscopic appearance

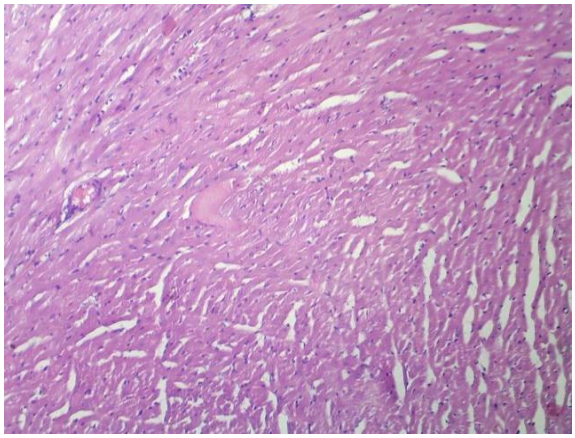
Section studies from the kidney show normal cortex and medulla. The glomeruli, interstitium and blood vessels are unremarkable. There is no evidence of inflammation and necrosis.

HEART

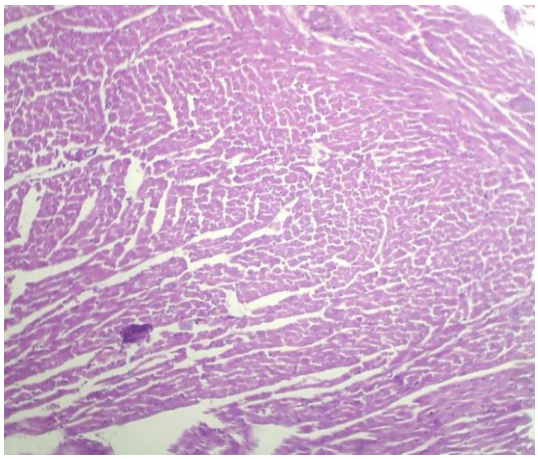
Figure 18:- T.S of Rat Heart Showing Normal Cells In Sub Acute Toxicity Studies of Extract



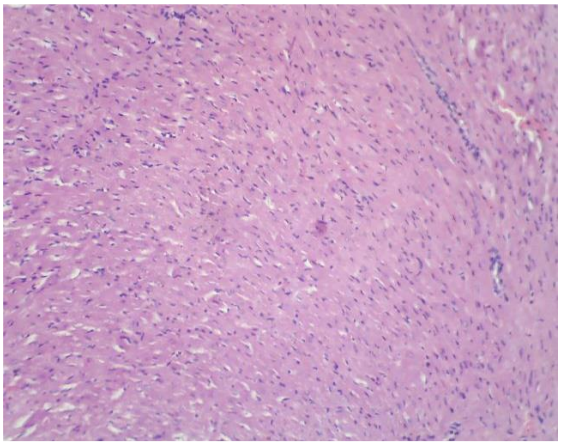
Control



Lower dose



Medium dose



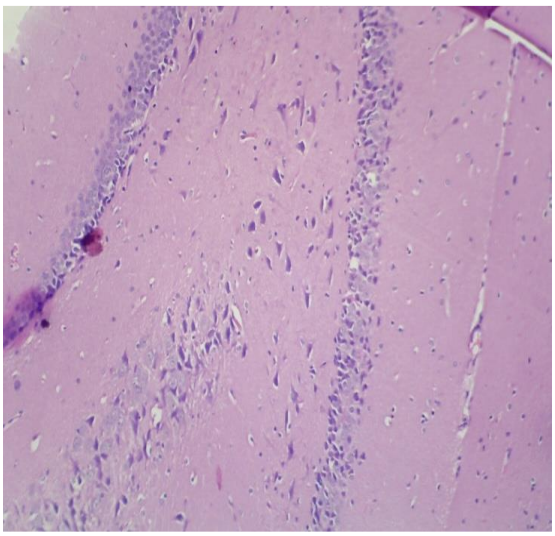
Higher dose

Microscopic appearance-

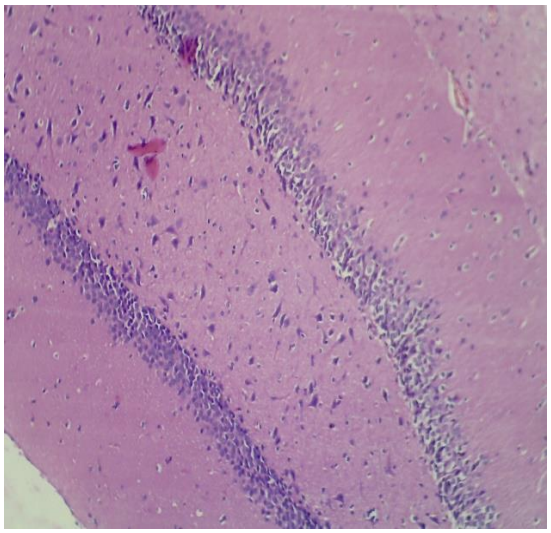
Section studies from the heart shows normal myocardium with myocytes. There is no evidence of myocytic degeneration or edema.

BRAIN

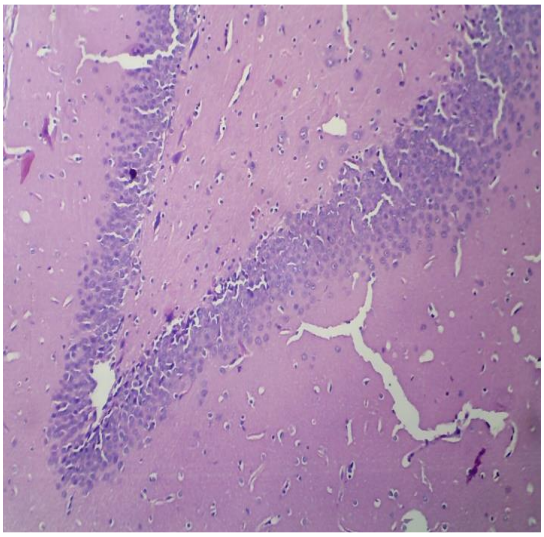
Figure 19- T.S of Rat Heart Showing Normal Cells In Sub Acute Toxicity Studies of Extract



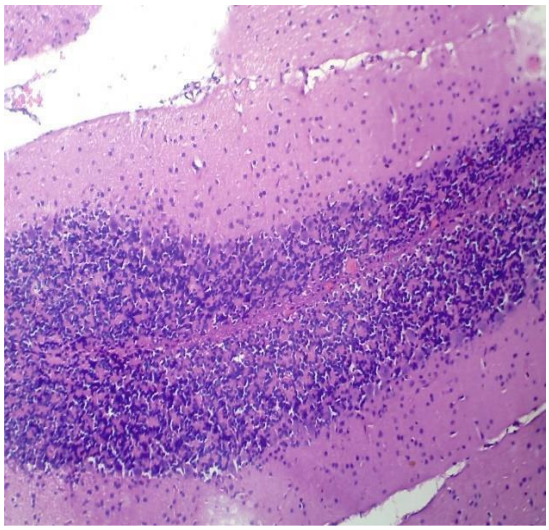
Control



Lower dose



Medium dose



Higher dose

Microscopic appearance-

Section studies from the brain shows normal cerebellum. Brain parenchyma, purkinjic cells and basal ganglion unremarkable. There is no evidence of inflammation and necrosis

DISSCUSION

In the sub-acute toxicity studies, no animal mortalities were observed in any of the study groups throughout the study period. The animals did not exhibit any treatment related abnormal behavioural traits. For all the dose level falling in the category NOAEL <100 mg/kg. The observations indicated that long-term administration of the extract had no adverse effects on the general health of the animals. No significant differences were observed in body weights or food consumption of the animals of the treatment groups when compared with that of the control groups. Similarly, no significant changes in haematology parameters and blood biochemistry parameters of the animals of the treatment groups when compared with that of the control groups. However, on completion of the treatment animals were sacrificed, necropsied and pathological examination of vital organs such as liver, heart, kidney and brain were performed and result showed that cells were within normal

ANTIDIABETIC ACTIVITY

Oral glucose tolerance test

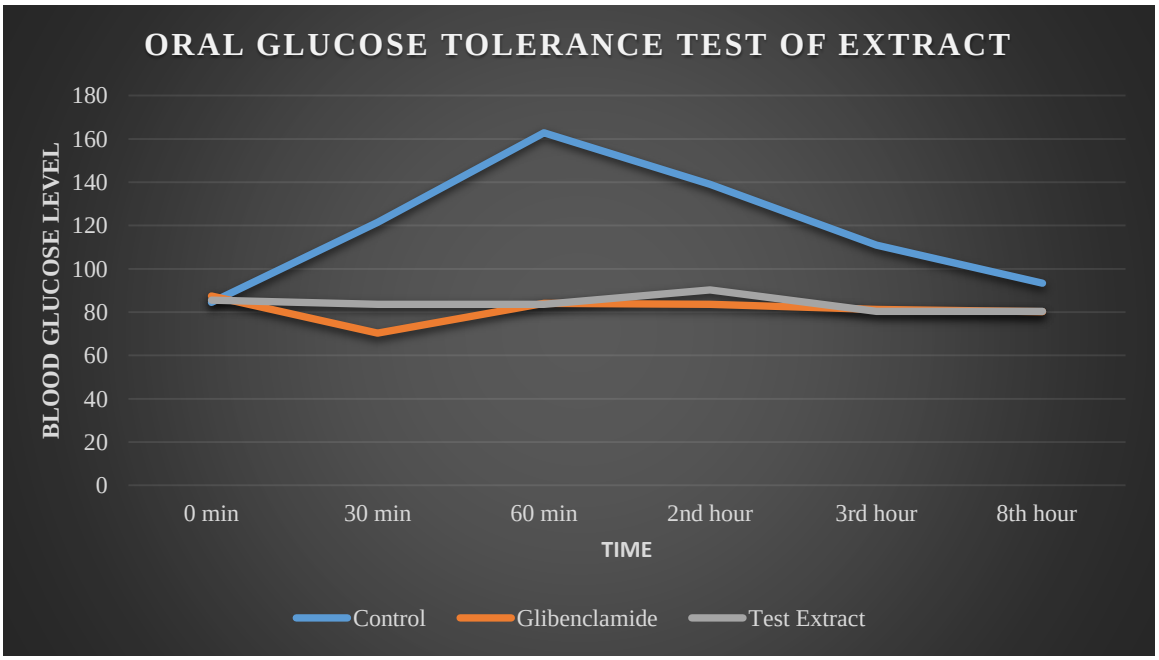
In the OGTT the extracts at a dose of 200mg/kg significantly reduced the blood glucose level at 30 minutes after glucose administration. Standard drug glibenclamide produced activity at all the time interval tested

TABLE 19:- ORAL GLUCOSE TOLERANCE TEST OF EXTRACT

Group	Blood glucose levels (mg/dl)					
	0 min	30 min	1st hour	2nd hour	3rd hour	8th hour
Control	84.5 ± 1.14	121.4 ± 1.52	162.91 ± 13.67	139 ± 2.074	111.02 ± 5.805	93.4 ± 1.304
Glibenclamide mg/kg	87.5 ± 10.164	70.3 ± 7.19	84.0 ± 10.271	83.6 ± 13.342	81.2 ± 7.791	80.2 ± 7.328
Test Extract 200mg/kg	85.6 ± 5.263	83.6 ± 6.894	83.6 ± 1.924	90.2 ± 2.408	80.4 ± 1.517	84.4 ± 2.302

All value are expressed as mean ± SEM (n=6)

Figure 20:- ORAL GLUCOSE TOLERANCE TEST OF EXTRACT



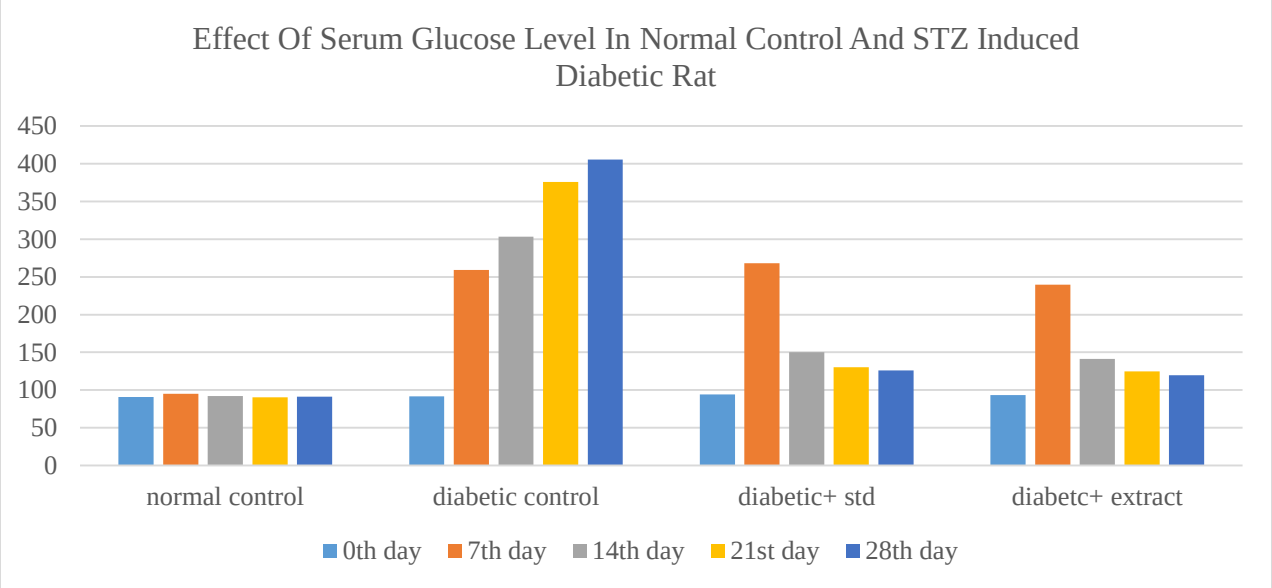
EFFECT OF EXTRACT ON SERUM GLUCOSE LEVEL

Table 20:- Effect of Extract on Serum Glucose Level in Normal Control and STZ Induced Diabetic Rats

S.NO	TREATMENT	SERUM GLUCOSE LEVEL				
		Initial	7th day	14th day	21stday	28th day
1	Normal control	90.9 ±1.47	94.9 ±1.47	92.2± 4.1	90.3± 2.13	91.2 ±2.16
2	Diabetic control	91.6 ±1.4	259.3 ±3.51	303.3 ±5.5	375.7 ±1.3	405.3 ±1.26
3	Diabetic+ Standard	94.1 ±1.2	267.9± 3.15	150.3 ±1.4	130.2 ±0.5	125.9 ±1.0
4	Diabetic+ Extract 200mg/kg	93.5 ±1.22	239.7 ±1.25	141.1 ±0.7	124.9 ±1.7	119.7 ±2.49

All values are expressed in MEAN ± SEM (n=6)

Figure 21:- Effect of Serum Glucose Level In Normal Control And STZ Induced Diabetic Rat



EFFECT OF EXTRACT ON BODY WEIGHT

There was gradual increase in body weight in normal control while the diabetic control continues to lose the weight. However treated diabetic group gained 6.25%, 8.24% as compared with the diabetic control and diabetic treated towards normal range. Extract changes in the body weight shows in the tables.

Table 21:-Effect Of Extract On Body Weight In Normal Control And STZ Induced Diabetic Rats.

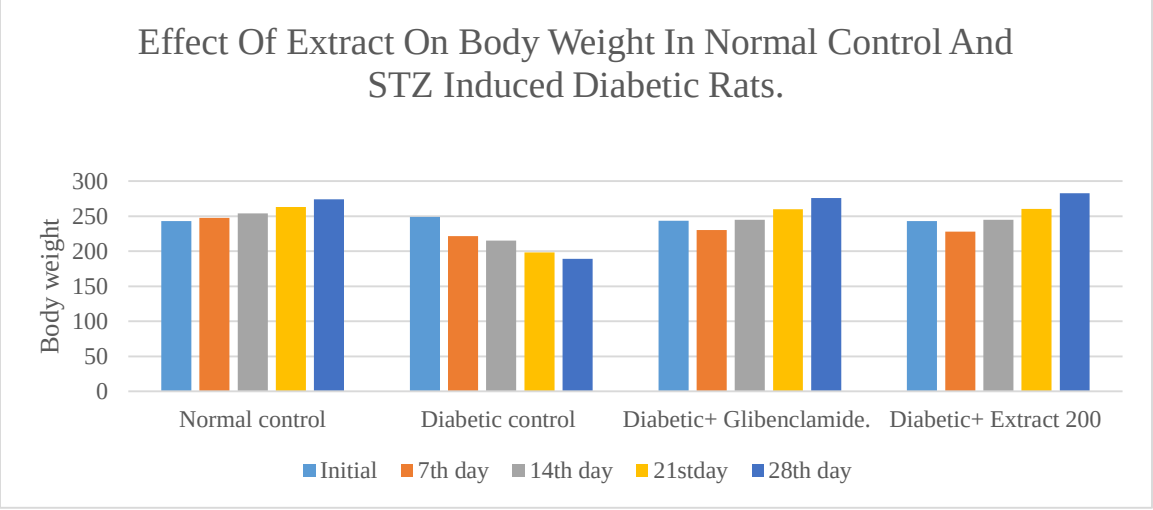
Sl No	TREATMENT	BODY WEIGHT				
		Initial	7th day	14th day	21stday	28th day
1	Normal control	242.9±3.5	247.8±1.33	254.1±2.333	263.11±8.5	274±1.95
2	Diabetic control	248.9±4	221.8±5.55	215.1±2.4	198.1±3.9	189±1.64
3	Diabetic+ Glibenclamide.	243.5±5	230.2±4.88	245.1±2.3	260.1±1.34	275.8±1.6
4	Diabetic+ Extract 200	243.2±25	228.1±4.6	245.1±3.4	260.5±6.7	282.8±2.7

All value are expressed mean ±SEM (n=6)

P<0.001, as compared to diabetic control

P<0.001 as compared to Normal control.

Figure 22:- Effect On Extract on Body Weight



8. SUMMARY AND CONCLUSION

Phytochemical screening

Qualitative Phytochemical screening and ethno botanical survey on the Artocarpus Hetrophyllus Leaf extracts the presented of certain phyto constitutions such, Proteins and amino acids, tannins, flavonoids, carbohydrates, glycosides, terpenoids.

Qualitative Phytochemical screening and ethno botanical survey on the Piper Nigrum Leaf extracts the presented of certain phyto constitutions such steroids, tannins, flavonoids, carbohydrates, alkaloids, terpenoids

The phytochemical constituents such as glycosides, tannins, alkaloids, triterpenoids, and flavonoids may be linked to the anti diabetic activity.

Acute toxicity studies

To check the safety profile of the combination of the leaf extract of Piper Nigrum and Artocarpus Hetrophyllus in the ratio of 1:1, it was subjected to the acute toxicity study which conformed the absence of any toxicity or mortality at the higher dose of 2000mg/kg. Thus the Extract can be classified as a safe drug category according to the Global harmonized Classification System quoted in the OECD guidelines 1996.

Based on the Toxicity studies 100mg/kg is used as a dose of extract and middle dose 200mg/kg and higher dose as 400mg/kg used for sub-acute toxicity studies.

Sub-acute toxicity studies

Sub-acute toxicity study was done and the results shows nontoxic nature for the Extract. Also, all the animals from control and all the treated groups up to dose 400 mg/kg survived throughout the dosing period of 28 days. Animals from all the treated groups exhibited comparable body weight gain with that of controls throughout the dosing period. No significant changes in the organ weight were observed. Furthermore no specific cell damage was noticed by the microscopic examination. The central and autonomic profiles were normal throughout the study and no specific alterations were noticed.

The results of the effect of the extract on the body weight of the animals compared with vehicle are as shown in **Table 15** and **Fig. 14**. There were no significant increases in the weight of

animals treated with 100 mg of extract However, there were little amount of weight reduction body weight in middle dose and higher dose treated animals.

The results of the effect of Extract on absolute organ weights of male and female rats are as shown in **Table 16**. Macroscopic examination did not show any changes in the colour of organs of the treated animals compared with vehicle. There were no significant changes in the relative weights of the liver, kidney and heart in both males and females. Treatment had no effect on spleen, stomach and testes of male rats.

Hematological analysis and Biochemical analysis conducted at the end of the dosing period revealed no abnormalities attributable to the treatment.

Effect of Extract on blood sugar level

Streptozotocin treatment will produce significant increase in serum glucose level with respective normal control group. The administration of Extract 200 mg/kg and glibenclamide 0.6 mg/kg significantly reversed the increase in serum glucose concentration in Streptozotocin induced rats. The extract changes in the serum glucose level are shown in the **table 20**.

Diabetic mellitus (DM) is an endocrine disorder in which the glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cells or because of inadequate release of insulin from the pancreatic cells of beta cells. The fundamental mechanism underlying hyperglycemia involved over population and decreases utilization of glucose by the tissue.

Streptozotocin a beta cytotoxic induces diabetic in a wide variety of animal species including rats by selectively damaging the insulin secreting beta cells of pancreas i.p injection of STZ produces fragmentation of DNA of beta cells of pancreas which stimulates poly (ADP ribose and deflects NDA ultimately leading of destruction of beta cells and it is evidenced by clinical symptoms of hyperglycemia**.**

Dose dependent of the effect of the glibenclamide showed rapid normalization of blood glucose due to its insulin releasing effects.

In our present study there was a significant weight gain Extract treated the diabetic rats compared with the normal control rats and this observation shows anabolic effect of the Extract on body weight on the diabetic rats.

Hyperglycemia and insulin resistance both seem to have important in the pathogenesis of macro vascular complications. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The hyperglycemia in the diabetes might inhibit tissue repair in the macro vascular beds. In the present study of Extract treated group shows hypoglycemic activity and it confirms the presence of the anti- diabetic activity. Sulfonylurea such as glibenclamide is often used as a standard drug in the STZ induced diabetic to compare to the efficacy of antihyperglycaemic compound. In study there was a significant elevation in blood glucose level in the diabetic control group as compared with normal animal. The Extract treated group exhibited significant reduction of fasting plasma glucose level as compared to the diabetic control group. Over production of glucose by means excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus.

Diabetes is a common chronic ailment for which the patient has to take insulin to maintain the blood sugar level. It is interesting to see how the Extract tackles this problem. It corrects the function of pancreas, stimulating it to produce insulin in the natural way, which in turns way to maintain the blood sugar level. Extract revitalizes and rejuvenates the organs, the dysfunction of which is causing the disease. This bring back normal functioning of the organs. It is also maintaining the healthy state body. Since no artificial chemical are involved, it doesn't cause any side effects

CONCLUSION

The presented study is an attempt to investigate the effect of ethanolic extract of the combination of the leaf extract of Piper Nigrum and Artocarpus Hetrophyllus in the ratio of 1:1 on Streptozotocin induced diabetic in albino rats.

The Phytochemical study was screening showed the presence of tannins, carbohydrate, Flavonoids and reducing sugar which is responsible for the anti-diabetic activity.

The animals were induced with STZ at a dose of 60mg/kg intraperatoneal and the diabetic animals were treated with Extract at a dose of 200mg/kg for 28 days orally. The serum glucose, body weight were measured which show significantly increased when compare with positive control group.

The finding of the presence investigation suggests the Extract has potential for its evaluation as protective agents against toxicity induced by Streptozotocin.

Mechanism of the extract is not completely established which may further done for protective action in the future studies

9. BIBLIOGRAPHY

REFERENCE

1. V. R. Kannan, G. S. Rajasekar, P. Rajesh, V. Balasubramanian, *et al.*, "Anti-diabetic activity on ethanolic extracts of fruits of terminalia chebula retz. Alloxan induced diabetic rats," *American Journal of Drug Discovery and Development*, 2012.
2. V. Jakus and N. Rietbrock, "Advanced glycation end-products and the progress of diabetic vascular complications," *Physiol. Res*, vol. 53, pp. 131-142, 2004.
3. P. K. Das, S. Bhattacharya, J. N. Pandey, and M. Biswas, "Antidiabetic activity of trapa natans fruit peel exctract against streptozotocin induced diabetic rats," *Global Journal of Pharmacology*, vol. 5, no. 3, pp. 186-190, 2011.
4. U. S. Sharma and A. Kumar, "Anti-diabetic effect of rubus ellipticus fruit extract in alloxan induced diabetic rats," *Journal of Diabetology*, vol. 2, no. 4, June 2011.
5. American Diabetes Association. Economic costs of diabetes in the US in 2002. Diabetes Care. 2003; 26: 917-932.
6. M. Velraj, M. Singh, V. Ravichandiran, S. Nirmala, and S. Ragala, "Antidiabetic activity of ethyl acetate and ethanolic extract of scindapsus officinalis fruit in alloxan induced diabetic rats," vol. 3, no. 3, pp. 1305-1310, July-Sept. 2011.
7. A. K. Datusalia, C. P. Dora, and S. Sharma, "Acute and chronic hypoglycemic activity of sida tiagii fruits in N5-streptozotocin diabetic rats," *Acta Poloniae Pharmaceutican Drug Research*, vol. 69, no. 4, pp. 699-706, 2012.
8. Marles RJ, Farnsworth N: Antidiabetic plants and their active constituents. Phytomedicine 1995, 2(2):137–189.
9. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology> Chapter 344. Diabetes Mellitus> Epidemiology
10. Global burden of diabetes. International Diabetes federation. Diabetic atlas fifth edition 201, Brussels. Yach D, Hawkes C, Gould CL, Hofman KJ (2004) The global burden of chronic diseases: overcoming impediments to prevention and control. JAMA 291: 2616 2622.
11. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053

12. American Diabetes Association (1998) Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 21: S5-S19.
13. Habtamu Wondifraw Baynest Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus Baynes J Diabetes Metab 2015, 6:5
14. Wareham N, O'Rahilly. The changing classification and diagnosis of diabetes. *British Medical Journal* 1998; 317-360
15. Samreen Riaz, Review on Diabetes mellitus. *Sci Res & Essay*. 2009,4 (5), 367-373
16. P. Jaya Preethi, "Herbal medicine for diabetes mellitus: A Review", International Journal of Phytopharmacy Vol. 3 (1), pp.01-22, Jan-Feb 2013
17. WHO Expert Committee on Definition (1999) Diagnosis and Classification of Diabetes Mellitus and its Complications, Geneva:1-59
18. Mayfield J (1998) Diagnosis and classification of diabetes mellitus: new criteria. Am Fam Physician 58: 1355-1362, 1369-70
19. American Diabetes Association. Economic consequences of diabetes mellitus in the U.S. in 1997. Diabetes Care 1998;21(2):296-309.
20. Adapted from Orr, DP. Contemporary management of adolescents with diabetes mellitus. Part 1: Type 1 diabetes. Adolescent Health Update 2000;12(2), Table 2, p 3
21. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology > Chapter 344. Diabetes Mellitus > Insulin Biosynthesis, Secretion, and Action
22. Patidar D (2011) Pharmacology- III. (2ndedtn). Meerut: Shree Sai Prakashan. 113 – 4.
23. American diabetes association.,"diagnosis and classification of diabetes Mellitus,"diabetes care, volume 36, supplement 1, january 2013
24. Al Homsi MF, Lukic ML (1992) An Update on the pathogenesis of Diabetes Mellitus, Department of Pathology and Medical Microbiology (Immunology Unit) Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.
25. Hussain AN, Vincent MT (2007) Type 1 Diabetes Mellitus
26. Raju SM, Raju B (2010) Illustrated medical biochemistry. 2nd Edition. Jaypee Brothers Medical Publishers ltd, New Delhi, India.
27. Holt RI (2004) Diagnosis, epidemiology and pathogenesis of diabetes mellitus: an update for psychiatrists. Br J Psychiatry Suppl 47: S55-63

28. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology > Chapter 344. Diabetes Mellitus >Pathogenesis
29. Defronzo RA, Ferrannini E (1988). Lily Lecture 1987. The Triumvirate: Beta Cell, Muscle, Liver. A Collusion Responsible for NIDDM. Diabetes. 37:667-687.
30. American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33 Suppl 1: S62-69.
31. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengård J, Kesäniemi YA (1992); Concordance for Type 1 (insulin dependent) and Type 2 (non insulin-dependent) diabetes mellitus in population based cohort of twins in Finland. Diabetologia, 35:1060-1067
32. .Mahler RJ, Adler ML (1999) Clinical review 102: Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab 84: 1165-1171
33. Sekikawa A, Tominaga M, Takahashi K, Eguchi H, Igarashi M, et al. (1993) Prevalence of diabetes and impaired glucose tolerance in Funagata area, Japan. Diabetes Care 16: 570-574
34. Botero D, Wolfsdorf JI (2005) Diabetes mellitus in children and adolescents. Arch Med Res 36: 281-290.
35. Holt G. I. (2004). Diagnosis, epidemiology and pathogenesis of diabetes mellitus an update for Psychiatrists. Br. J. Psychiatry. 184:s55- s63.
36. SE Kahn: JClin Endocrinol Metab 86:4047, 2001; RN Bergman, M Ader: Trends Endocrinol Metab 11:351, 2000
37. Stang J, Story M (eds) Guidelines for Adolescent Nutrition Services (2005)
38. Botero D, Wolfsdorf JI (2005) Diabetes mellitus in children and adolescents. Arch Med Res 36: 281-290.
39. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology > Chapter 344. Diabetes Mellitus > Acute Complications of DM
40. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology > Chapter 344. Diabetes Mellitus > Chronic Complications of DM
41. Gillett MJ (2009) International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: Diabetes Care 2009; 32(7): 1327-1334. Clin Biochem Rev 30: 197-200.

42. Guidance for Industry, Diabetes Mellitus: Developing Drugs and Therapeutic Biologics for Treatment and Prevention, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). 2008: 3.
43. Health Canada website. www.hc-sc.gc.ca. Accessed February 28, 2013
44. A. McGibbon et al. / Can J Diabetes 37 (2013) S56-S60
45. Harrison's Online > Part 16. Endocrinology and Metabolism > Section 1. Endocrinology > Chapter 344. Diabetes Mellitus > Long-Term Treatment
46. Bolen S, Feldman L, Vassy J, et al. Systematic Review: Comparative Effectiveness and Safety of Oral Medications for Type 2 Diabetes Mellitus. Ann Intern Med. 2007 July 16; [Epub ahead of print]
47. Carroll MF, Izard A, Riboni K, et al. Control of postprandial hyperglycemia: optimal use of short-acting insulin secretagogues. Diabetes Care. 2002 Dec;25(12):2147-52.
48. Colagiuri S, Cull CA, Holman RR, UKPDS Group: Are lower fasting plasma glucose levels at diagnosis of type 2 diabetes associated with improved outcomes? Diabetes Care 25:1410-1417, 200
49. Rosenstock J, Hassman DR, Madder RD, et al. Repaglinide versus Nateglinide Monotherapy: a randomized, multicenter study. Diabetes Care 27:1265-1270, 2004
50. Rana Ibrahim,"diabetes mellitus type ii: review of oral treatment options",International Journal of Pharmacy and Pharmaceutical Sciences Vol 2, Suppl 1, 2010
51. . Waxler-Morrison NE. Plural medicine in Sri Lanka: Do Ayurvedic and Western medical practice differ, Soc Sci Med 1988; 27: 531-44.
52. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal Plants and Natural Products (1994–98), Indian J Pharmacol. 2000;32:S81–S118
53. Dandiya PC, Bapna JS. Pharmacological research in India, Ann Rev Pharmacol 1974; 14:115-126.
54. Adithan C. Pharmacological research in India, 1972-1995 - An analysis based on IPS conferences. Indian J Pharmacol 1996; 28:125-128.
55. Singh H. Steady decline in clinical pharmacology research in India - A decade trend-analysis of IJP research publications (1990-1999). Abstracts of XXXIII annual conference of IPS, 2000. Indian J Pharmacol 2001; 33:51-70.

56. Eddouks M, Maghrani M. Phlorizin-like effect of *Fraxinus excelsior* in normal and diabetic rats. *J Ethnopharmacol* 2004;9:149-54.
57. Kesari AN, Kesari S, Santosh KS, Rajesh KG, Geeta W. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. *J Ethnopharmacol* 2007;112(2):305-11.
58. Latha M, Pari L. Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clin Exp Pharmacol Physiol* 2003;30(1-2):38-43.
59. Subbulakshmi G, Naik M. Indigenous foods in the treatment of diabetes mellitus. *Bombay Hospital J*. 2001;43(4):548-61.
60. Sabu MC, Subburaju T. Effect of *Cassia auriculata* Linn. On serum glucose level, glucose utilization by isolated rat hemidiaphragm. *J Ethnopharmacol* 2002;80(2-3):203-6.
61. Shrabana C, Tuhin KB, Begum R, Liaquat A, Mosihuzzaman M, Nilufer N, Azad KAK, Biswapati M. Advanced studies on the hypoglycemic effect of *Caesalpinia bonducella* F. in type 1 and 2 diabetes in Long Evans rats. *J Ethnopharmacol* 2003;84:41-6.
62. Daniel SF, Norman RF. The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environ Health Perspect* 2001;109:69-75.
63. Michael PK, Asim AB, Robert SB. The Utility of Oral Diabetes Medications in Type 2 Diabetes of the Young. *Curr Diab Rev* 2005;1:83-92.
64. Pulok KM, Kuntal M, Kakali M, Peter JH. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006;106:1–28.
65. Mohamed B, Abderrahim Z, Hassane M, Abdelhafid T, Abdelkhaleq L. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). Int J Diabetes Metabol 2006;14:1-25.
66. Manisha Modak, Priyanjali Dixit, Jayant Londhe, Saroj Ghaskadbi, and Thomas Paul A. Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes., J. Clin. Biochem. Nutr.2007; 40: 163–173.
67. Jarald E, Joshi SB, Jain DC: Diabetes & Herbal Medicines: Review Article. Iranian Journal of Pharmacology & Therapeutics 2008; 7: 97-106.

68. Nazli Shahin, Sanjar Alam, Mohammad Ali.,"Pharmacognostical Standardisation and Antidiabetic activity of Artocarpus Heterophyllus Leaves Lam," *International Journal of Drug Development & Research January-March 2012, Vol. 4 Issue 1, ISSN 0975-9344*
69. Jayaweera, D. M. A. (1982). Medicinal plants used in Ceylon 4-89. Part 11, National Science Council: Sri Lanka, Colombo. 4-89.
70. Fernando, M. R., Wickramasinghe, S. M. D. N., Thabrew, M. I., Ariyaratne, P. L. and Karunanayake, E. H., (1991) *Journal of Ethnophamocology* **31,** 277-282
71. Chandrika U. G, Wedage W. S., Wickramasinghe S. M. D. N And Fernando W. S., "Hypoglycaemic Action Of The Flavonoid Fraction Of *Artocarpus Heterophyllus* Leaf", *Afr. J. Trad. Cam* (2006) 3 (2): 42 – 50
72. Sureka Chackrewarthy and M.I. Thabrew.,"Hypoglycaemic and Hypolipidaemic Effects of an Ethylacetate Fractionof *Artocarpus heterophyllus* Leaves
73. Haidy S. Omar, Hesham A. El-Beshbishy, Ziad Moussa, Kamilia F. Taha, and Abdel Nasser B. Singab., *2011.* Antioxidant Activity of *Artocarpus heterophyllus* Lam. (Jack Fruit) Leaf Extracts: Remarkable Attenuations of Hyperglycemia and Hyperlipidemia in Streptozotocin-Diabetic Rats. *TheScientificWorldJOURNAL (2011) 11, 788–800*
74. Onyesife, Chioma O, Ogugua, Victor N. And Anaduaka, Emeka G., 2014. Hypoglycemic Potentials of Ethanol Leaves Extract Of Black Pepper (*Piper Nigrum*) On Alloxan-Induced Diabetic Rats. Annals of Biological Research, 2014, 5 (6):26-31
75. Shubham atal, rajendra p. Agrawal, savita vyas, pradeep phadnis And niket rai.," evaluation of the effect of piperine *per se* on blood glucose Level in alloxan-induced diabetic mice",acta poloniae pharmaceutica ñ drug research, vol. 69 no. 5 pp. 965-969, 2012
76. M. Kaleem, Sheema, h. Sarmad and B. Bano. Protective effects of *Piper Nigrum* and *Vinca Rosea* in Alloxan induced Diabetic Rats. Indian J Physiol Pharmacol 2005; 49 (1): 65–71.
77. Grover n, bafna p.a., rana a.c., "diabetes and methods to induce experimental diabetes", international journal of pharmacy and biological sciences volume 1, issue 4, oct-dec 2011 414-419
78. Etuk, E.U., "Animals models for studying diabetes mellitus" Agric. Biol. J. N. Am., 2010, 1(2): 130-134
79. Mythili, M.D., Vyas, R., Akila, G., Gunasekaran, S., 2004. Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microscopy Research and Technique 63, 274–281.

80. Haider Raza and Annie John.," Streptozotocin-Induced Cytotoxicity, Oxidative Stress and Mitochondrial Dysfunction in Human Hepatoma HepG2 Cells" *Int. J. Mol. Sci.* 2012, *13*, 5751-5767
81. Nazli Shahin, Sanjar Alam, Mohammad Ali., " Pharmacognostical Standardisation and Antidiabetic activity of Artocarpus Heterophyllus Leaves Lam," International Journal of Drug Development & Research, January-March 2012 Vol. 4 Issue 1
82. Chandrika U. G, Wedage W. S., Wickramasinghe S. M. D. N and Fernando W. S., 2006. Hypoglycaemic Action of the Flavonoid Fraction of *Artocarpus Heterophyllus* Leaf. *Afr. J. Traditional*, Complementary and Alternative Medicines (2006) 3 (2): 42 – 50
83. Om Prakash, Jyoti, Amit Kumar, Pawan Kumar., 2013. Screening of Analgesic and Immunomodulator activity of *Artocarpus heterophyllus* Lam. Leaves (Jackfruit) in Mice. Journal of Pharmacognosy and Phytochemistry Vol. 1 No. 6 2013, 33-36
84. Efrilia Tanjung, Iskandar, Khairina, and Eko Suhartono.,"Antidiabetic and Antioxidant Activity of Jackfruit (Artocarpus Heterophyllus) Extract,"*Journal of Medical and Bioengineering Vol. 4, No. 4, August 2015*
85. Meshram R. L, Umbarkar S.N.,"Comparative evaluation for in vitro Antioxidant activity from Artocarpus heterophyllus Lamk and Balanites aegyptiaca L," International Journal of PharmTech Research Vol.3, No.4, pp 2006-2010, Oct-Dec 2011
86. Sindhu.S.Nair, Vaibhavi Kavrekar, Anshu Mishra., "Evaluation of In Vitro Anti diabetic Activity of Selected Plant Extracts," International Journal of Pharmaceutical Science Invention Volume 2 Issue 4 April 2013 PP.12-19
87. Anjali Ruikar, Rasika Torane, Ashwini Misar, Arvind Mujumdar, Vedavati Puranik, Nirmala Deshpande., "Antioxidant screening of a Medicinal Plant *Artocarpus heterophyllus,"* Int. J. Pharm. Sci. Rev. Res., 33(1), July – August 2015; Article No. 08, Pages: 35-36
88. Haidy S. Omar, Hesham A. El-Beshbishy, Ziad Moussa, Kamilia F. Taha, and Abdel Nasser B. Singab., *2011.* Antioxidant Activity of *Artocarpus heterophyllus* Lam. (Jack Fruit) Leaf Extracts: Remarkable Attenuations of Hyperglycemia and Hyperlipidemia in Streptozotocin-Diabetic Rats. *The Scientific World JOURNAL (2011) 11, 788–800*

89. M. Kaleem, Sheema, H. Sarmad and B. Bano., 2005. Protective Effects Of Piper Nigrum And Vinca Rosea In Alloxan Induced Diabetic Rats. Indian J Physiol Pharmacol 2005; 49 (1): 65–71
90. Onyesife, Chioma O, Ogugua, Victor N. And Anaduaka, Emeka G., 2014. Hypoglycemic Potentials of Ethanol Leaves Extract of Black Pepper (*Piper Nigrum*) On Alloxan-Induced Diabetic Rats. Annals of Biological Research, 2014, 5 (6):26-31
91. Shanmugapriya K, Peer Mohammed S, Saravana PS., "Evaluation of Phytochemical Constituents and Pharmacological Activities of Ethanolic Extract of *Piper nigrum* Leaves Against DLA in Swiss Albino Mice," International Journal of Pharmacognosy and Phytochemical Research 2014; 6(1); 27-38
92. Gayatri Nahak and R.K. Sahu., "Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum,"* Journal of Applied Pharmaceutical Science 01 (08); 2011: 153-157
93. K Shanmugapriya, Saravana P S, Harsha Payal, S Peer Mohammed and Binnie Williams., "Antioxidant potential of pepper (Piper nigrum Linn) leaves and its antimicrobial potential against some pathogenic microbes," Indian Journal of Natural Products and Resources Vol. 3(4) December 2012 pp 570-577
94. O. Onyesife Chioma, N. Ogugua Victor and G. Anaduaka Emeka., "Toxicological Assessments of Piper nigrum on Alloxan Induced Diabetic Rats," American-Eurasian Journal of Toxicological Sciences 6 (2): 30-34, 2014
95. Divya Pingili, Sneha Jijabapu Anarthe, Nulgumnalli Manjunathaiah Raghavendra., "Evaluation of the polyherbal extract for antioxidant, anticancer and antidiabetic activity" Annals of Phytomedicine 1(1): 39-45, 2012
96. Shubham Atal, Rajendra P. Agrawal, Savita Vyas, Pradeep Phadnis and Niket Rai.,"Evaluation of the Effect of Piperine *per Se* on Blood Glucose Level In Alloxan-Induced Diabetic Mice," Acta Poloniae Pharmaceutica - Drug Research, Vol. 69 No. 5 Pp.965-969, 2012
97. Onyesife, Chioma O, Ogugua, Victor N. And Anaduaka, Emeka G., "Ethanol Extract of *Piper nigrum* Leaves Ameliorates Serum Lipid Peroxidation and Antioxidant Enzymes Activities in Diabetic Rats" World Applied Sciences Journal 32 (1): 27-33, 2014

98. Popi Patilaya, Pazilah Ibrahim, and Zhari Ismail., " Effects of Standardized Fractions of *Piper nigrum* on the growth of *Mycobacterium tuberculosis* cells" Online International Journal of Medicinal Plant Research Volume 1, Issue 1, pp. 6-12; October 2012
99. A.Dinakar,P.Dwarakanadha Reddy,D.Swarnalatha,R.Pradeep Kumar, K.Alekya,S.Prasad Kumar, D.Vijay Kumar,A.Bharath ,M.Ravindar ., "Inhibition Of Thioacetamide –Induced Liver Fibrosis By Piper Nigrum Linn" Journal Of Global Trends In Pharmaceutical Sciences Vol.1, issue 1, pp. 1-8, October–December 2010
100. S.K. Shiva Rani, Neeti Saxena and Udaysree, "Antimicrobial Activity of Black Pepper (*Piper nigrum L.*)" Global Journal of Pharmacology 7 (1): 87-90, 2013
101. Kaleem M, Medha P, Ahmed Q U, Asif M, Bano B., "Beneficial effects of *Annona squamosal* extract in streptozotocin-induced diabetic rats" Singapore Med J 2008; 49 (10) : 800
102. Shaik Abdul Nabi, Ramesh Babu Kasetti, Swapna Sirasanagandla, Thandaiah Krishna Tilak, Malaka Venkateshwarulu Jyothi Kumar and Chippada Appa Rao., "Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats" BMC Complementary and Alternative Medicine 2013,
103. C. Hari Kumar, Dr. J.N. Suresh Kumar, B. Mohammed Ishaq, G. Usha Rani, Dr. K. Vanitha Prkash., "Antidiabetic Activity Of A Polyherbal Preparation.," *Pharmacologyonline* 2: 780-787 (2010)
104. Prakash O, Kumar R, Mishra A, Gupta R. *Artocarpus heterophyllus* (Jackfruit): An overview. Phcog Rev 2009;3:353-8
105. A.V. Rama Rao, Mala Varadan and Venkataraman. Colouring matter of the *A. hetetophyllus. Indian J. Chem.* 11: 298-299 (1973).
106. Perkin and Cope. The constituents of *Artocarpus integrifolia. J. Chem. Soc.* 67: 937-944 (1895).
107. G. Pavanasasivam, M. Uvais and S. Sultanbawa. Cycloartenyl acetate, cycloartenol and cycloartenone in the bark of *Artocarpus species. Phytochemistry,* 12(11): 2725-2726 (1973).
108. F.A. Chawdhary, M.A. Raman. Distribution of free sugars and Fatty acids in Jackfruit. *Food chemistry.* 60(1): 25-28 (1997).

109. B.R. Barik, T. Bhaumik, A.K. and A.B. Kundu. Triterpenoids of *Artocarpus heterophyllus, J. Indian Chemical Soc.* 74: 163-164 (1997).

110. Chun-Nan Lin and Chai-Ming Lu. Heterophylol, a phenolic compound with novel skeleton from *Artocarpus heterophyllus, Tetrahedron letters.* 34(17): 8249-8250 (1993).

111. M.C. Nath, 1937b. *Z Physiology Chem.* 247, 9.Quoted in Plant, and Chaturvedi, K. *Phytochemistry* 28: 2197-2199 (1989).

112. R. Dayal and T.R. Seshadri. Colourless compounds of the roots of *Artocarpus heterophyllus.* Isolation of new compound artoflavone. *Indian J Chem.* 12: 895-896 (1974).

113. G. Suresh Kumar, P.S. Appukttan and D.K. Basu a- D-Galactose - specific lectin from jack fruit seed. J. *Biosci*. 4: 257-261 (1982).

114. G. Pereira-da-Slva, AN. Moreno, F. Marques, C. Oliver, MC. Jamur, A. Panunto-Castelo, MC. Roque-Barreira, Neutrophil activation induced by the lectin KM+ involves binding to CXCR2. *Biochim. Biophys. Acta*. 1: 86-94 (2006).

115. Chai-Ming Lu and Chun-Nan Lin. Two 2~, 4', 6'--trioxygenated flavanones from *Artocarpus heterophyllus. Natural Products Research Center* 33(4): 909-911 (1993).

116. Chun-Nan Lin, Chai-Ming Lu and Pao-Lin Huang. Flavonoids from *Artocarpus heterophyllus. Phytochemistry.* 39(6): 1447-1451 (1995).

117. K. Venkataraman. Wood of phenolics in the chemotaxonomy of the Moraceae, *Phytochemistry* 11(5): 1571-1586 (2001).

118. U.G. Chandrika, E.R. Jansz and N.D. Warnasuriya. Analysis of carotenoids in ripe jackfruit *(Artocarpus heterophyllus)* kernel and study of their bioconversion in rats. *Journal of the Science of Food and Agriculture* 85(2): 186 -190 (2004).

119. P. P. Hemborn. Contact therapy practiced by Mundas Chotanagar (Bihar). *Ethanobotany.* 8: 36-39 (1996).

120. M. Sato and S. Fujiwara. Flavones with Antibacterial activity against carcinogenic bacteria. J. *Ethnopharmacol.* 54(2-3): 171-176 (1996).

121. V. M. Vaidya Gogte, *Ayurvedic Pharmacology y and therapeutic use of medicinal plants,* (Swami Prakashananda Ayurvedic Research center, Mumbai, 2000) 656-657.

122. K. Gupta and N. Tandon, *Review on Indian Medicinal Plants,* (Indian Council of Medical Research, New Delhi, 2004) 182-200.

123. Meghwal M, Goswami TK (2012) Nutritional Constituent of Black Pepper as Medicinal Molecules: A Review. 1: 129.
124. Murthy CT, Bhattacharya S (2008) Cryogenic grinding of black pepper. J Food Eng 85:18-28.
125. Agbor GA, Vinson JA, Oben JE, Ngogang JY (2006) Comparative analysis of the *in vitro* antioxidant activity of white and black pepper. Nutrition Research 26: 659-663.
126. Ahluwalia VK, Raghav S (1997) Comprihensive experimental chemistry. New Age International Publishers, New Delhi.
127. Vijayan KK, Thampuran RVA (2000) Pharmacology, toxicology and clinical applications of black pepper. Harwood Academic Publishers.
128. Jain K (2006) Holistic health healing and astrosciences. An international source book, B Jain Publishers, New Delhi.
129. Harbone JB. Phytochemical methods. London: Chapman & Hill, 1973.
130. Harbone JB. Phytochemistry. London: Academic Press, 1993.
131. Trease GE, Evans WC. Pharmacognosy (13th ed.). London: Bailliere Tindall Ltd., 1989.
132. OECD guideline for testing of chemicals. Acute toxicity studies 423
133. David E.Tupper and Robert B. Wallace. Utility of the neurological examination in rats. Acta neurobiol. Elw. 1980, 40 : 999-1003
134. Gad.S.C,An Approach to the Design and Analysis of Screening Studies in Toxicology.International Journal of ToxicologyMarch/April 1988 7: 127-138.
135. Moser VC, Tilson HA, MacPhail RC, Becking GC, Cuomo V, Frantik E, et al. 1997e. The IPCS Collaborative Study on Neurobehavioral Screening Methods: II. Protocol design and testing procedures. Neurotoxicology 18:929–938.
136. Naik SR, mandlik RV, Desai Sk. Antidiabetic activity of a polyherbal formulation (DRF/AY/5001). Indian J Exp Biol 2008; 46:599-606.
137. Kuttan R, Joy KL, Anti-diabetic activity of Picrorrhiza kurroa extract, J Ethnopharmacol 1999; 167: 143-8.
138. Crofton, K. M. (1992). Reflex modification and the Assessment of Sensory Dysfunction. In: Neurotoxicology (H. A. Tilson and C. L. Mitchell, Eds.), pp. 181-212, Raven Press, New York

139. MEYER, J. L., AND E. T. SCHULTZ. 1985. Migrating haemulid fishes as a source of nutrients and organic matter on coral reefs. Limnol. Oceanogr. 30: 146-156.

140. Karunanayake EH, Hearse D J, Mellows G. The metabolic fate and elimination of streptozocin. Biochemical Society Transactions 1975; 3: 410-14. 7.

WEBOGRAPHY

a) http://www.myvmc.com/diseases/diabetesmellitustype1insulindependentjuvenileonset
b) http://www.aafp.org/afp/2001/0501/p1747.html
c) http://www.iloveindia.com/indian-herbs/herbs-for-diabetes accessed on 01/12/12.
d) http://dx.doi.org/10.5772/52538
e) http://plants.usda.gov/core/profile?symbol=arhe2
f) http://www.ntbg.org/plants/plant_details.php?plantid=1219
g) https://plants.usda.gov/java/ClassificationServlet?source=display&classid=PINI3
h) http://www.kew.org/science-conservation/plants-fungi/piper-nigrum-black-pepper
i) http://dx.doi.org/10.5772/52538